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FRANK J. ORLAND

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Organized: New York City, Dec. 10, 1920. Of those at the meeting of organization, the following remain active members: Adolph Berger, Theodor Blum, G. W. Clapp, H. S. Dunning, A. H. Merritt, B. B. Palmer, L. M. Waugh.

Objects (Const., Art. II): The association has been established to promote broadly the advancement of research in all branches of dental science and in the related phases of the sciences that contribute directly to the development of oral health service, and as a whole; and further, to encourage and facilitate cooperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology to the end that dentistry may render cumulatively more perfect service to humanity.

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Endowment fund of the International Association for Dental Research (as of March, 1956): $1,802.34. There have been no expenditures from this fund.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
37th General Meeting
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March 19-22, 1959
For further instructions, contact Dr. Burrill, Sec.-Treas.

THE JOURNAL OF DENTAL RESEARCH

This JOURNAL is an official publication of the International Association for Dental Research. It is dedicated to the dissemination of knowledge pertaining to the oral cavity, its contents and contiguous parts, and its relation to and effect on the total human organism. The JOURNAL publishes original investigations in these and related fields. Articles are accepted only if they are contributed solely to this JOURNAL.

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2. *General Policy.*—The policy of the JOURNAL OF DENTAL RESEARCH is to submit all manuscripts to appropriate referees for evaluation. The Constitution of the International Association for Dental Research provides for an Advisory Editorial Board to assist the Editor in this regard. A manuscript submitted to the Editor will be promptly acknowledged and later, upon proper review, will be (a) accepted, (b) returned for revision, or (c) rejected.

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Following a concise introduction, a manuscript should, where possible, be divided into the following parts: Materials and Methods, or Experimental Methods; Results; Discussion; Summary; Acknowledgments; References.

The title of the paper should be followed by the authors' names and professional addresses. Academic degrees will not be published since these usually can be ascertained from a biographical directory. Change of authors' address since writing should be placed in a footnote. Reference to research grants, which should include its full title and number, also must be made in a footnote.

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5. *References.*—References should be listed at the end of the article in the order in which they appear in the text and with a number corresponding to a superscript in the text. References should take the following form: Name of author(s) with initials following surname, full title of paper, name of journal in accepted abbreviated form, volume number, first page of paper, year. References to books should include the author's name, title of the book, edition, place of publication, year of publication, and publisher. Examples:

1. (To a periodical) Ware, B. E.: Dental Disease, *J. D. Res.* **33**: 889, 1954.
2. (To a book) Heck, O.: Oral Problems, ed. 2, Chicago, 1950, Y. B. Good & Sons, Ch. 2, p. 114.

The accepted abbreviated form for dental journals is that used in "Index to Dental Literature," compiled by the American Dental Association. For other journals, the usage in the "List of Periodicals Abstracted by Chemical Abstracts," should be followed. Authors should consult current issues of the JOURNAL for further details covering the accepted form.

Works cited in the text by reference to their authors should in the first instance be referred to with the names of all authors. Subsequent mention may be abbreviated to "and associates."

References cited should be restricted to closely pertinent papers. Articles cited in the references should be either "accepted for publication" in a specific journal or already printed.

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Names of proprietary substances and trade names should be capitalized and, where possible and appropriate, should be accompanied by their generic or chemical names. Source of supply where required should be referred to in a footnote.

Microorganisms should be referred to in accordance with the International Rules of Nomenclature. When applicable, the nomenclature for bacteria presented in *Bergey's Manual of Determinative Bacteriology*, 7th edition, 1957, will be followed.

8. *Annotations.*—Manuscripts submitted as short research notes must be not more than 500 words in length. If illustrative material is submitted, the text should be correspondingly shorter since only one page is allowed to each article. These notes should present results of original work together with adequate data. Contributors should follow the format and bibliographical form used for Annotations in current issues of the JOURNAL.—F. J. O.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
ANNOUNCEMENT OF 1959 GENERAL MEETING

The 37th General Meeting of the International Association for Dental Research will convene at the Sheraton-Palace, San Francisco, California, on Thursday, Friday, Saturday, and Sunday, March 19, 20, 21, and 22, 1959. The Dental Materials Group of the Association will meet on Friday, Saturday, and Sunday, March 20, 21, and 22. The American Association of Dental Schools will meet at the same place beginning on Sunday, March 22.

Notice to Prospective Program Participants.—Abstracts of items for the program must be limited to 250 words *including* title, authors' names, etc. Three copies are required. *For further instructions, contact Dr. Burrill, Secretary-Treasurer.* Nonmembers are invited to submit items for possible presentation on the program. Sponsorship by a member is requested in such cases. Abstracts of papers on the program will be published in the JOURNAL OF DENTAL RESEARCH *after* the meeting. The members have been polled for an expression of opinion on whether or not the abstracts should also be preprinted, and they voted to have no preprinted abstracts with a January first deadline.

By Council action at the 1958 meeting, the Read-by-Title section has been eliminated from the program for the 1959 meeting.

Abstracts entered in the *Edward H. Hatton* Award Competition should conform to the specifications published in the JOURNAL OF DENTAL RESEARCH 33: 590, 1954. Entries should be clearly marked as such.

DAN Y. BURRILL, SECRETARY-TREASURER
Northwestern University Dental School
311 E. Chicago Avenue
Chicago 11, Illinois

EDITORIAL VIEWS AND NEWS

Because it is not always easy to compose an initial sentence for a manuscript, there follows a brief expository essay on this timely topic by Gerald J. Cox of the University of Pittsburgh:

The Opening Sentence.—The very first sentence of a scientific essay, including this one, represents the most difficult hurdle that faces the man who believes he has something to say and decides to enshrine it in permanent form. The opening sentence can only be avoided by remaining silent, but that is frequently a greater difficulty. It may be suspected, however, that many have remained silent because they have not been able to cross the Rubicon. Thus the world has been cheated of much pent-up wisdom by the failure of many would-be authors to launch their flow of words. The opening cannot be taken care of later. Any sentence used just to get under way will likely contain some of the execrable elements peculiar to the opening sentence. Thus when the real first line is prefaced to the preliminary opening, the final work will be twice as bad. In fact, by writing the last sentence first and working back systematically, it is possible to produce the very worst possible essay. The only way is to write the first sentence and then do the best to save the rest of the composition.

The whole tenor of an essay is sometimes given by the first sentence; sometimes only one word, but more frequently a phrase, reveals the warped mind of the author. One goes on only in hope that the remainder cannot be as bad. It does, in fact, seldom become worse. "Look for the bias in the opener" is the reader's equivalent of "Cherchez la femme." Other vagaries are kept for other parts of the essay though it would indeed be fortunate if all bad things could be crowded into one sentence so that the wise reader could avoid it and proceed. But alas it cannot be that way. Atrocities must be left for other lines and particularly for the last sentence which competes vigorously with the first to be last in excellence.

I have not infrequently been appalled by an opening such as "It is universally agreed that ...," and though I might go along with "It is now admitted on all sides that dental caries is due to the action of bacteria" (Goadby, K., Brit. M. J. 2: 769, 1910), there may still remain, 48 years after that opener appeared, many who have their teeth decay with a belief in some other cause. In fact, goaded by Goadby, I am going to conclude by writing a first sentence that within 48 years may be followed by a supporting essay: "Dental caries may now be completely prevented by proper nutrition during the formation of each and every tooth." —G. J. C.

Grant Award for the Journal.—An official notification has just been received that the Surgeon General approved, upon the recommendation of the National Advisory Dental Research Council, a grant (D-880) of U. S. Public Health Service funds for publication of research manuscripts in the JOURNAL OF DENTAL RESEARCH.

Final Results of J. D. Res. Questionnaire.—A fairly large number of opinions were received late, after results of the poll were announced in the August issue. The total count at this time stands as 278 in favor, 55 not in favor, and 25 undecided about the short rapidly published notes. (The first several of such research notes appear in this issue.) There were 283 in favor of an informative lead abstract instead of a terminal summary and 48 against the matter with 26 undecided. Biographical sketches of members to appear in the JOURNAL were favored by 157 in contrast to 135 unfavorable and 65 undecided votes.

Instructions to Prospective Authors.—After some contemplation, it was decided both time and effort could be saved if specific instructions were made available to authors regarding manuscript preparation. Such a discussion appears in this issue of the JOURNAL.

—FRANK J. ORLAND
EDITOR

DENTAL PAPILLA IN TISSUE CULTURE

MITIO NIIZIMA* AND MARTIN CATTONI**

Department of Anatomy, Tissue Culture Laboratory, The University of Texas, Medical Branch, Galveston, Texas

INTRODUCTION

SEVERAL workers have been engaged in tissue culture studies of dental tissues, especially in relation to developmental stages. The majority of previous works have dealt with organ culture of tooth germs. The pioneering work of Glasstone[1, 2, 3] was one of the first organized efforts in the use of organ culture of tooth germ for in vitro studies. Others cultured extirpated dental papilla or young pulp. Pinkerton and Boyle[4] have shown that the behavior of connective tissue from tooth germs of newborn kittens was similar to that of connective tissue from other locations except that, in certain transplants, long cell processes were seen. Cells from the odontoblastic layer seem to retain their peculiar characteristics of developing long cytoplasmatic processes even after several transplantations. Szabo[5] reports a study in which extirpated pulp from unerupted molars and incisors of the mouse gave rise to fibroblastic outgrowths and some wandering cells. Lefkowitz and co-workers[6, 7] studied the growth and development of the rat molar in embryos of controlled age and described a detailed technic for the dissection of tooth germs. To the authors' knowledge, there is no description regarding the initial stage of outgrowth of pulp cells in vitro. The purpose of the present work is to observe the outgrowth pattern from cultivated dental papilla of developing tooth germs in the living state.

MATERIALS AND METHODS

Dental pulp from tooth germs of newborn kittens and 4½-month-old human and 20-day-old rat fetuses was used. At these stages of development (Fig. 1), it was not difficult to extirpate the dental pulp from the concavity of the enamel organ. This was done using sharpened watchmaker's forceps under a binocular dissection microscope. The extirpated pulp was cut at the proximal end and separated from other components of the germ which lay outside the enamel organ. In the permanent canine of newborn kittens (Fig. 1) or deciduous teeth of the human fetus, the dentin cap was already formed on the tip of

This investigation was supported by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contracts No. DA-49-007-MD-447 and No. DA-49-007-MD-32, respectively.

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the pulp. In such cases, the pulp was explanted either with or without the dentin cap. In some instances, a small amount of enamel organ, mostly the inner enamel epithelium, was intentionally left attached to the surface of the dentin cap in order to examine the behavior of the two kinds of tissues when they came in contact with each other. Co-explantation of dental pulp and enamel epithelium was carried out in the same manner.

The roller tube and hanging-drop methods were employed for culture. The plasma clot which held the explants to the cover slips consisted of one drop of rooster plasma and one drop of embryo extract from 8-day-old chicks. In the hanging-drop culture, one drop of nutrient fluid was added on the surface of the clot after its coagulation. In the roller tube method, the clot was made on a 12 by 50 mm. cover glass, and placed into a 16 by 150 mm. test tube. The clot was allowed to coagulate for 1 or 2 hours before adding 2 ml. of the fluid nutrient. The fluid nutrient consisted of 50 per cent human ascitic fluid of malignant origin, 45 per cent Gey's balanced salt solution, and 5 per cent chick embryonic extract. Penicillin, was added to make a final concentration of 1,000 units per milliliter. The initial pH of the nutrient fluid was approximately 8.4 as indicated by phenol red. Cultures were incubated at 37° C. The period of cultivation ranged from several hours to 54 days. In the roller tube method, explants more often showed a tendency to detach from the cover slips than the other types of tissues. Therefore, the routine hanging-drop method was more suitable for the present work, especially for observation of initial status of cell outgrowth from the explants, since this method allowed high power microscopy without remounting the cultures.

The cultures were examined frequently with the light microscope during cultivation and suitable specimens were selected for phase contrast microscopy in the living state. Still pictures and time lapse cinematography were taken by this method to reveal more precise structure and behavior of the living cells. Fixed and stained preparations were also made for routine histologic examination.

RESULTS

A well-defined basement membrane between the dental pulp and inner enamel epithelium was observed. At the beginning of the formation of dentin, the basement membrane covered the outer surface of the dentin as the dentino-enamel membrane. When dental pulp was dissected from the enamel organ, this membrane was usually left on the surface of the dental pulp (Fig. 2). However, at times it was injured during manipulation. Examination of a stained section of extirpated pulp showed these facts clearly (Figs. 3 *a, b, c*). Patterns of outgrowth differed according to the conditions on the surface of the explants.

In some explants, outgrowth began within 18 hours. Needle-like cell processes from the natural surface of the pulp appeared in the first outgrowth (Figs. 4, 5, 6). When the surface was intact, in other words, when the basement membrane was perfect, the outgrowth of the cell processes seemed to be

delayed or sometimes completely suppressed. The outgrowing processes were either single or ramified. They reached considerable length in a relatively short period of time. In 48 hours, some of them were 220 μ in length. The processes usually appeared thread-like, but were often flattened and sometimes showed nodes, which might possibly be a sign of degeneration of the processes. Here

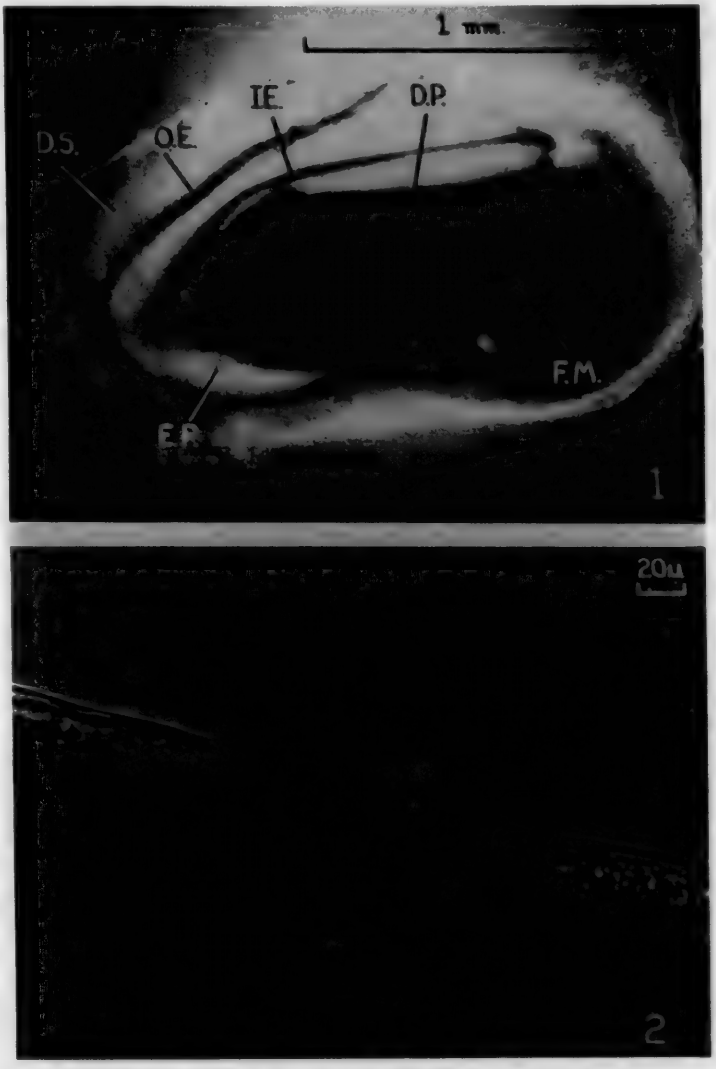

Fig. 1.—Tooth germ of a lower permanent canine of a newborn kitten; stained with May-Grünwald-Giemsa and made transparent with xylol. *D. P.*—dental pulp; *D. S.*—dental sac; *E. P.*—enamel pulp; *F. M.*—free margin of the enamel organ; *I. E.*—inner enamel epithelium; *O. E.*—outer enamel epithelium.

Fig. 2.—Surface of dental pulp of a lower permanent canine of a newborn kitten. Observed with light microscope in toto in living state.

and there, they contained optically dense granules which were common in the cell body. These processes showed no active movement. In motion picture sequences, little movement was discernible except during emigration. In the area where needle-like cell processes grew abundantly, outgrowth of the cell body itself was somewhat retarded. Fig. 7 shows another field of the same

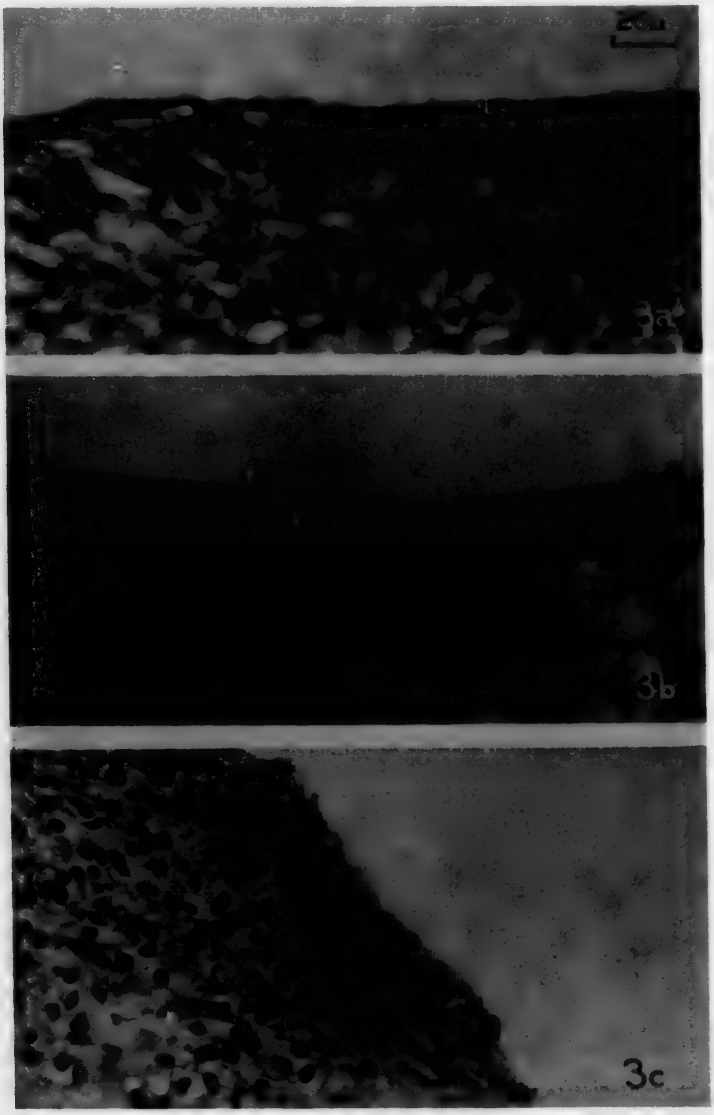

Fig. 3.—Section of dental pulp of a lower permanent canine (7 μ thick) showing condition of the surface of explants. Newborn kitten. (Azan stain.) *a*—Surface covered with the basement membrane. *b*—Surface whose basement membrane was removed. *c*—Cut surface.

preparation as that represented in Fig. 6, but made 2 days later. The outgrowing cells were mostly spindle-shaped and contained long slender processes. They usually appeared at the surface of the pulp where no apparent injury was recognized except for the removal of the basement membrane. Cell bodies appeared to migrate from the explant following the long processes.

In contrast, cell outgrowth from the slightly injured surface of the pulp presented a different aspect. The outgrowth started in 3 days. Emigrating cells

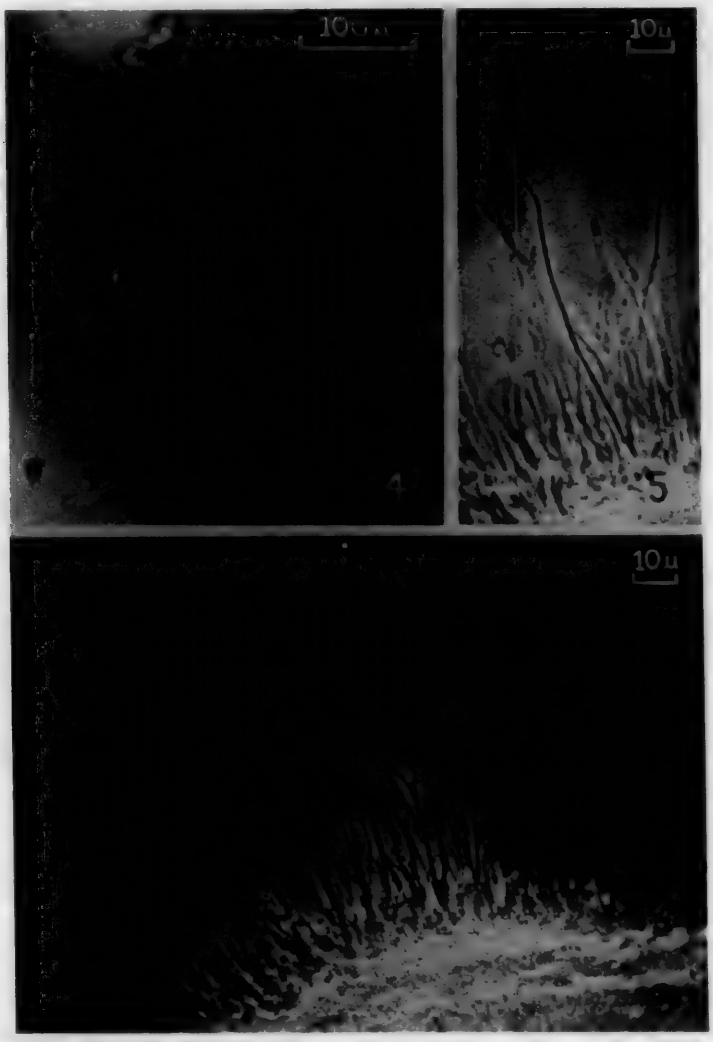

Fig. 4.—Dental pulp. Human fetus. One-day culture. Light microscopy of living preparation.

Fig. 5.—Dental pulp. Human fetus. Two-day culture. Dark phase contrast microscopy of living preparation showing a long cell process.

Fig. 6.—Same field as Fig. 5 at different level of focus.

were at first spindle-shaped and crowded, standing perpendicular to the surface of the explant (Fig. 8). Sometimes, they formed additional dense sheets of spindle cells (Fig. 9). In moving picture sequences, active movement of macrophages was observed along the course of the spindle cells. Macrophages were also seen prior to outgrowth of any other cellular elements (Fig. 10). From surfaces where a considerable amount of tissue had been eliminated, stellate cells were seen emigrating instead of spindle cells (Fig. 11). Patterns

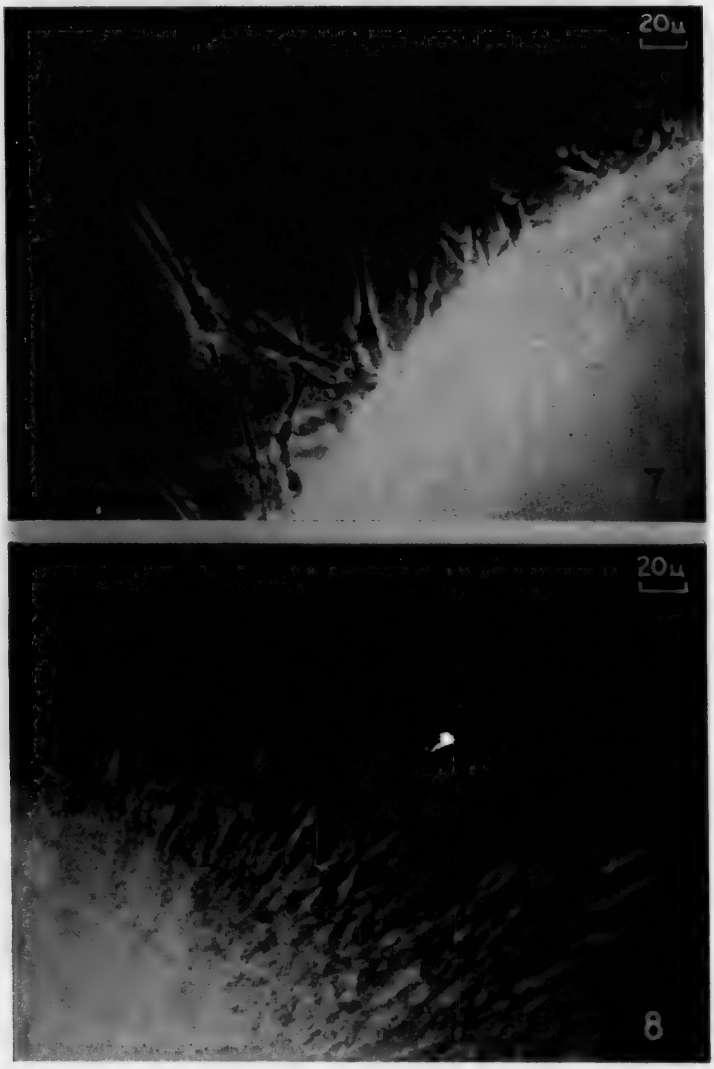

Fig. 7.—Cell outgrowth from intact surface of dental pulp. Human fetus. Three-day culture. Dark phase contrast microscopy.

Fig. 8.—Cell outgrowth from injured surface of dental pulp. Same culture as Fig. 7.

of outgrowth from the cut surface differed according to tissue conditions. Often, there was no outgrowth. However, occasionally an abundant migration of stellate and spindle cells took place (Fig. 12).

When epithelial element was co-explanted with dental pulp, both epithelial and fibroblastic outgrowths were seen. In such cases, the fibroblastic outgrowth from the pulp tissue was more vigorous than in solitary explantation, and connective tissue cells spread on the epithelial sheet which was derived from the

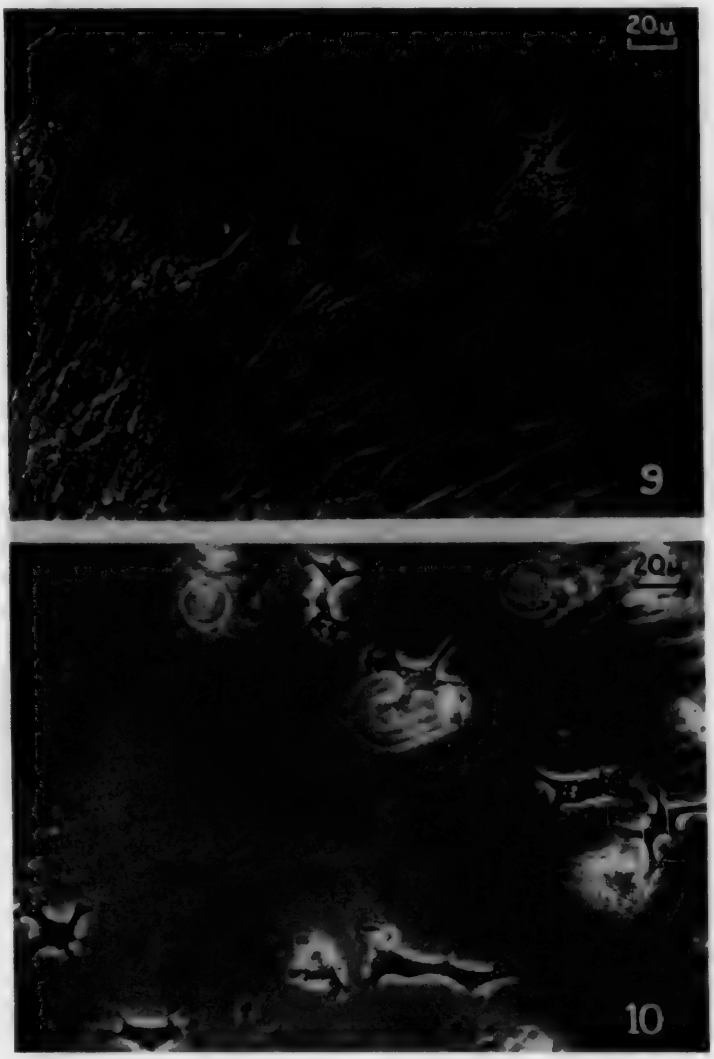

Fig. 9.—Cell outgrowth from dental pulp. Newborn kitten. Six-day culture. Dark phase contrast microscopy.

Fig. 10.—Wandering cells from an explant of dental pulp. Rat embryo. Two-day culture. Dark phase contrast microscopy.

enamel epithelium. Emigration of the cells from the pulp explant was continuous so that the original explant became almost depleted, leaving its dentin cap (Fig. 13).

In the area of cell outgrowth, invasion of capillary blood vessels from the explant was common (Fig. 14). Since the blood vessels grew out after dense fibroblastic and epithelial sheets were already formed, they were overlooked

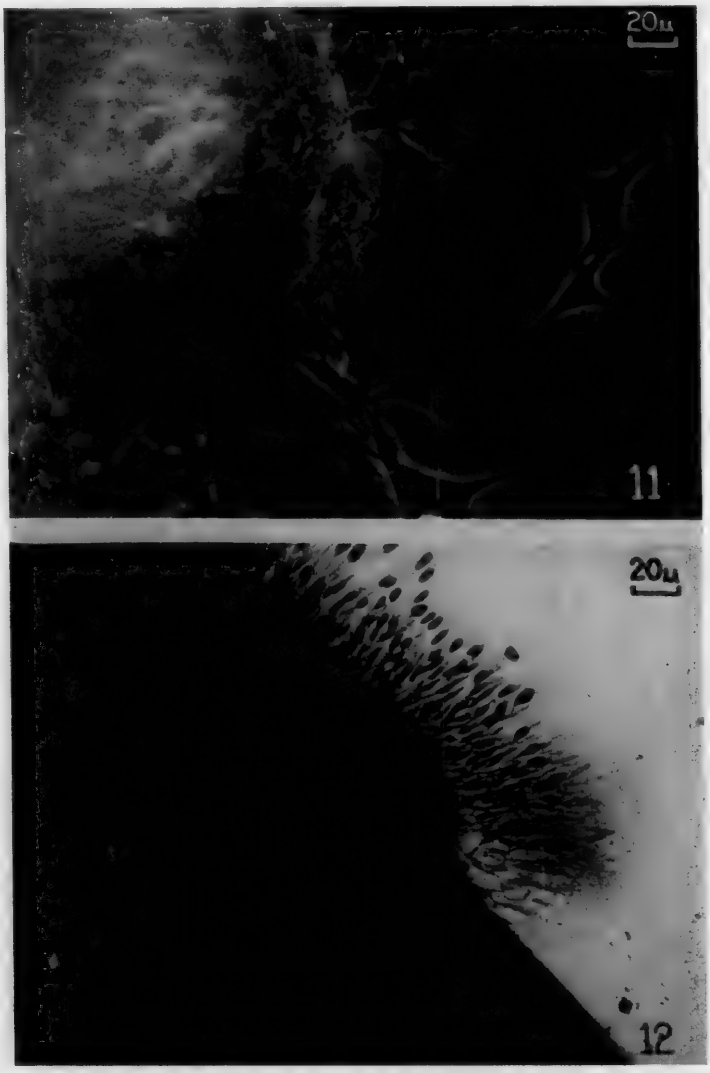

Fig. 11.—Cell outgrowth from injured surface of dental pulp. Newborn kitten. Six-day culture. Dark phase contrast microscopy.

Fig. 12.—Dental pulp showing difference of pattern of outgrowth from both injured and intact surface of the explant. Human fetus. Six-day culture. (Jacobson's stain.)

in the living state in making gross inspection through the curved wall of roller tubes. Hence, there were no records made of these structures in the living state. The vessels were found in the preparations which were fixed on the third and sixth days of culture. In the preparations stained with Heidenhain's "Azan" the blood vessels were continuous with those in the original explants of the dental pulp. They formed a network of capillaries which consisted of a single

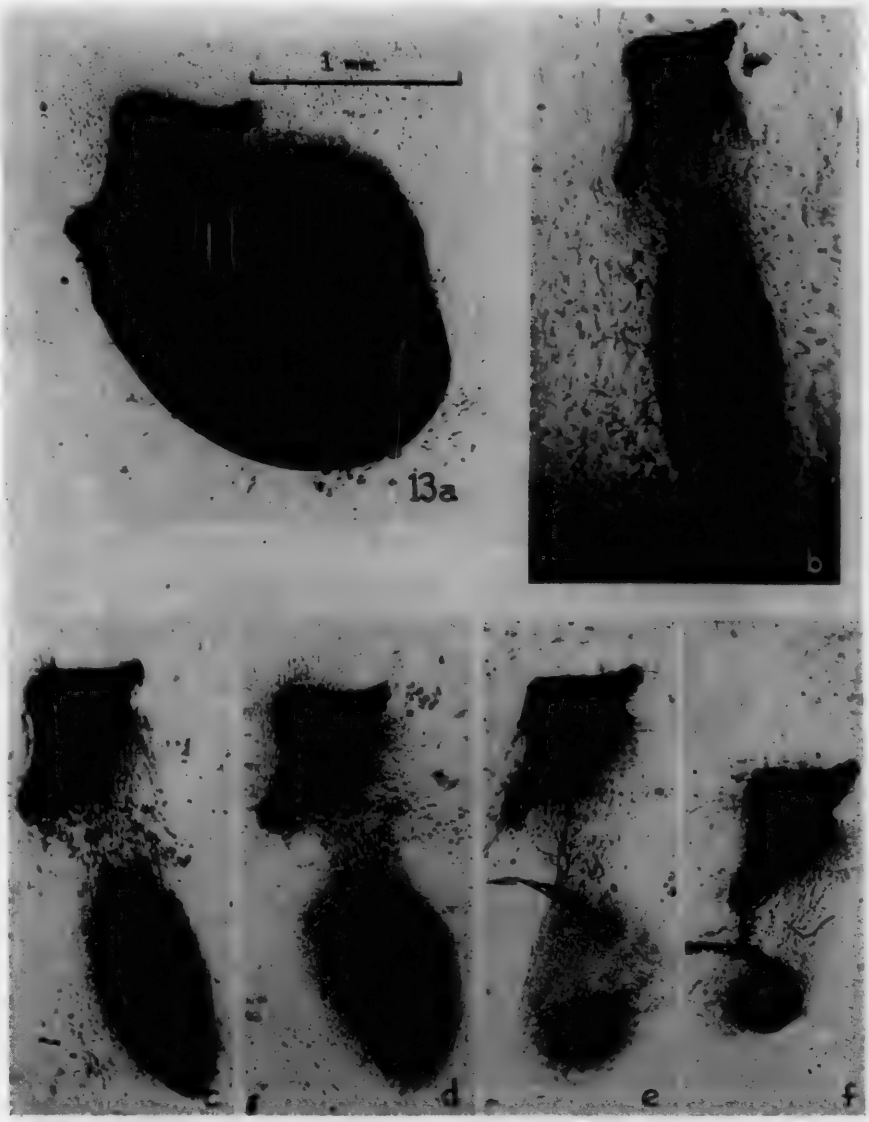

Fig. 13.—Dental pulp explanted together with enamel epithelium. Human fetus. *a*—10-day culture. *b*—21-day culture. *c*—28-day culture. *d*—33-day culture. *e*—41-day culture. *f*—54-day culture.

layer of endothelium whose branching sprouts seem to have dense sharp tips of cytoplasm. Red blood cells were occasionally present in the lumen, sometimes in large numbers and were also seen outside of the vessels. White blood cells were scarce. Even if the pulp tissue of the original explant was disorganized, and became hardly stainable, blood vessels in the explant still maintained good stainability (Fig. 15).

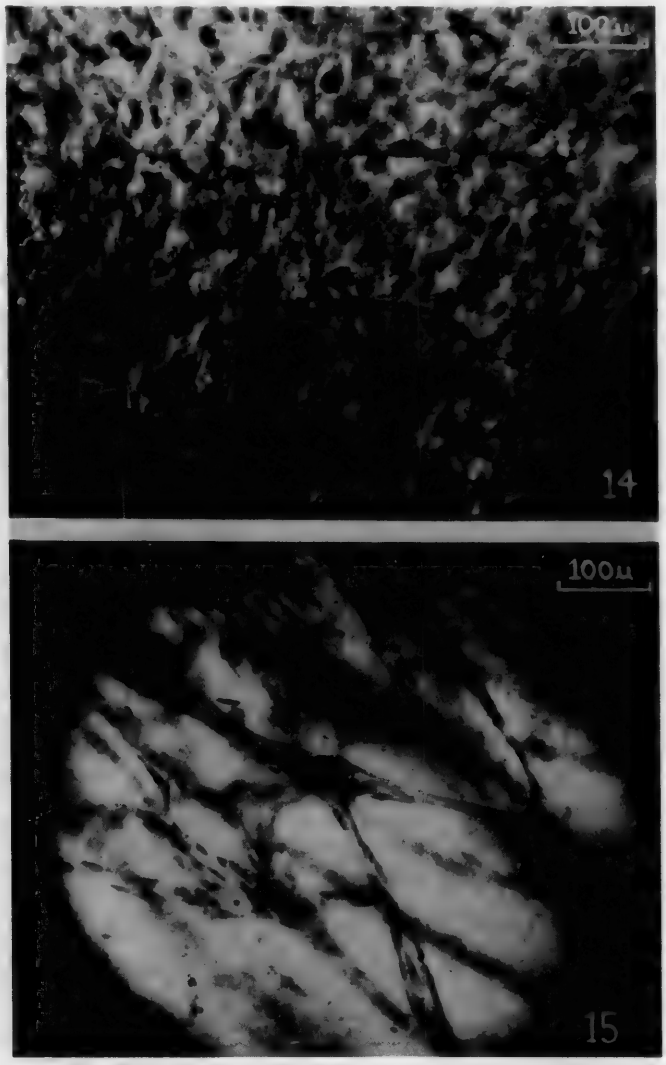

Fig. 14.—Blood vessels in outgrowth of a permanent canine from dental pulp. Newborn kitten. Six-day culture. (Azan stain.)

Fig. 15.—Blood vessels which were not disorganized in the area of an explant which degenerated. Newborn kitten. (Jacobson's stain.)

Co-explantation of enamel epithelium and dental pulp was attempted in order to observe the behavior of these two types of tissues when they came in contact with each other. Formation of the enamel needs contact of the dental pulp with the enamel epithelium. In moving picture sequences, several arrangements were observed. When connective tissue cells from the dental pulp approached the inner enamel epithelium in an open space, long cell processes were extended from spindle cells to the undulating membrane of the epithelial cells. They almost reached the edge of these elements and then retracted. This phenomenon was repeated several times. The two types of cells did not always come in contact. On the other hand, in explants where both kinds of cells were put in close proximity at the onset, different results were obtained. Continuous association of the connective tissue cells with enamel epithelium was established. They were joined by means of intercellular bridges. Both kinds of cells were clearly distinguishable due to slight differences in the optical density of the cytoplasm; epithelial cells being darker than connective tissue cells. The density changed in the middle of the connecting bridges. No formation of mineralized structures was found between these tissues under the condition of the present investigation.

DISCUSSION

Although the outgrowth pattern of cells from the dental pulp in vitro generally has been observed in fixed and stained preparations, we have had little knowledge as to the exact origin of emigrating cells. Therefore, little is known as to whether there is a distinct difference in behavior between odontoblasts and ordinary pulp cells in vitro, expect that odontoblasts presumably have longer cytoplasmic processes than ordinary pulp cells.

Outgrowth of the cell processes, which correspond to Tomes's fibers of odontoblasts, was repressed when the basement membrane was left intact. When the surface was slightly injured, i.e., when only the basement membrane was removed, the characteristic feature was the outgrowth of needle-like processes.

Cell outgrowth was rather poor. A considerable number of cytoplasmic processes, which connect odontoblasts closely with each other, seemed to delay wandering of these cells, building again a barrier against migration of the other pulp cells.

Szabo[5] reported, on the basis of his sectioned preparations of the explanted pulp, that the odontoblasts at the distal part of the pulp remained in their original position and that there was no growth of fibroblastic cells, although they increased underneath the odontoblastic layer.

At times, the cell bodies of odontoblasts loosened and consequently sporadic wandering of these cells could be seen (see Fig. 7). The cytoplasmic processes from the outer surface of the pulp and from the wandering odontoblasts themselves were always long. Emigrating cells from the considerably injured surface sometimes appeared spindle-shaped or stellate. A spindle shape was seen when cells were crowded and jostled one another. They did not have long cell processes. Hence, it became clear that odontoblasts, as Pinkerton and Boyle[4]

pointed out, have a tendency to grow long, slender processes while the other pulp cells produce either spindle- or stellate-shaped processes depending on various conditions. In the culture of extracted adult human pulp, we have previously found spindle cells with long, slender processes which may be identified as odontoblasts.

Fig. 12 shows clearly the contrast of cell outgrowth from intact and injured surfaces. From the proximal cut edge of the pulp, there was at times no cell outgrowth, but occasionally tremendous amounts of emigration of spindle and stellate cells could be seen. This seemed to depend upon mechanical trauma, especially that occasioned by twisting the tissue at the time of cutting.

Co-existence of epithelium seemed to accelerate emigration of connective tissue cells from the pulp explant. Widespread fibroblastic sheets were not unusual when the enamel epithelium was co-explanted and formed a sheet in the neighborhood of the dental pulp. Sometimes outgrowth of blood vessels was discernible. Growth of capillaries from the explant in vitro has been reported by several workers for various kinds of tissues, such as meso- and metanephros (chick embryo, Rienhoff[8]), liver (human embryo, Benevolenskaia[9]), skin (chick embryo, Lewis[10]), limb-bud (chick embryo, Scriba[11]), lung and mesonephros (chick embryo, Törö[12]), rib (rat fetus, Murray and Stout[13]). White and Parshley[14] obtained growth of blood vessels from bone marrow of adult chicken. Recently, Woodard and Pomerat[15] succeeded in growing patent blood vessels from adult human rib marrow. However, we have found no report of outgrowth of blood vessels from dental pulp in vitro. Since dental pulp is a highly vascularized tissue, it was to be expected that we would obtain capillary structures in vitro.

SUMMARY

1. Dental pulp from tooth germs of newborn kittens and 4½ month-old human and 20-day-old rat fetuses was cultivated in roller tubes and hanging-drop preparations in order to observe cell outgrowths from the explants in the living state.

2. Patterns of outgrowth from the explanted dental pulp differed according to conditions on the surface of the explants.

3. When the surface of the pulp was perfectly intact, i.e., the surface was left covered with the basement membrane, there was only a small amount of cell emigration.

4. When the basement membrane was removed without any severe injury of the pulp tissue, outgrowth of dentinal fibers from odontoblasts, migration of odontoblasts themselves and of some wandering cells was observed.

5. When the odontoblastic layer was eliminated, spindle and/or stellate cells with wandering cells emigrated from this area. Tremendous emigration of cells was sometimes seen in such preparations unless there was mechanical trauma.

6. Outgrowth of blood capillaries from dental pulp was observed in the culture of permanent canines from newborn kittens.

7. Enamel epithelium and connective tissue cells from dental pulp were in direct contact with each other by means of intercellular bridges.

Grateful acknowledgment is made to Dr. C. M. Pomerat for making available the facilities of his laboratory and for his helpful criticism of the manuscript.

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IN VITRO CHEMICAL AND BACTERIAL CHANGES IN SALIVA

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CHEMICAL changes in stagnating saliva, such as protein hydrolysis, indole, sulfide, lactic acid and ammonia formation, have been reported by various investigators.[1-3] Although it is accepted that microorganisms indigenous to the oral cavity bring about these chemical changes, there is no clear information on hand to show what microorganisms are involved.

The purpose of the present study was to determine the fate of certain representative types of the oral microflora in saliva incubated under various experimental conditions. In addition, the formation of ammonia and indole was also followed as a measure of microbial decomposition of nitrogenous constituents of saliva.

MATERIALS AND METHODS

Saliva collected by paraffin stimulation was stored at 1° C. until incubated. The saliva samples were incubated at 37° C. in either sterile screw-cap bottles or rubber-stoppered Erlenmeyer flasks.

The groups of organisms chosen for study were the lactobacilli, streptococci, Veillonella, and fusiform bacteria, since they are known to be present in large numbers in saliva and selective media were available for their enumeration. SL agar medium[4] was used for the enumeration of lactobacilli, FM agar medium[5] for fusiforms, CVI agar medium[6] for streptococci, and V_{12} medium[7] for Veillonella. Duplicate plates were prepared per determination.

Ammonia was determined by nesslerization after carrying out the Conway diffusion technic.[8] Total nitrogen was measured by the micro-Kjeldahl method. Total carbohydrate was measured as glucose by using anthrone reagent.[9] Indole determinations were carried out by modifying the method of Wood, Gunsalus, and Umbreit,[10] replacing the toluene extraction procedure by treating an equal volume of saliva with ethyl alcohol, chilling and centrifuging to obtain a clear supernatant fluid. Dry weights were determined on saliva samples which were dried to constant weight at 105° C.

RESULTS

Results of preliminary experiments showed that when whole saliva was incubated, the lactobacilli, streptococci, and Veillonella decreased with time.

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The fusiforms, on the other hand, increased. The addition of glucose to incubating salivas promoted an increase in the Veillonella and, to a lesser extent, in the streptococcal and lactobacillus populations. In a subsequent, more detailed experiment, the results generally confirmed preliminary observations and hence are reported here in detail.

The saliva pool from 7 individuals was divided into 6 lots. Sterile glucose solution was added to lots 1 and 2 to make 0.2 and 0.5 per cent final concentrations, respectively. Lots 3 and 4 received sterile sodium lactate solution

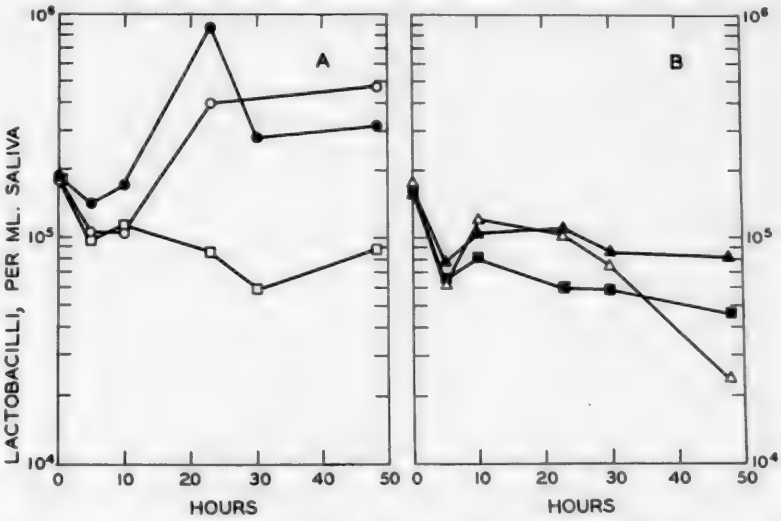

Fig. 1.—*A* and *B*, Effects of glucose, sodium lactate, and pH on lactobacillus populations in incubated saliva.
0.2% glucose—○ 0.2% sodium lactate △ pH 6.5 □
0.5% glucose—● 0.5% sodium lactate ▲ pH 7.7 ■

to make final concentrations of 0.2 and 0.5 per cent, respectively. The pH of lot 5 was adjusted to 6.5 with sterile HCl solution, whereas lot 6 was not altered (pH 7.7). Since the change in volume by the addition of glucose, sodium lactate, or HCl solutions was 1 per cent or less, no concentration corrections were made for the various determinations. The salivas were incubated for a period of 54 hours.

In Fig. 1, *A*, it is evident that the addition of glucose stimulated a great increase in the lactobacilli, whereas the effects of pH 6.5, 7.7, and lactate (Fig. 1, *A* and *B*) were not significantly different from each other. In the case of the fusiforms, the addition of glucose resulted in their rapid decline, in contrast to the other treatments (Fig. 2, *A* and *B*). The streptococcal population increased with the addition of glucose (Fig. 3, *A*), whereas the addition of lactate and the lowered pH (Fig. 3, *A* and *B*) maintained the population for a longer period than unaltered (pH 7.7) saliva. The Veillonella increased very rapidly in the

presence of sodium lactate (Fig. 4, *B*). At pH 7.7 saliva (Fig. 4, *B*), the Veillonella also showed the same initial rate of growth as with lactate, but not to the same extent. The 0.2 per cent glucose lot showed a slower rise but to a greater extent in the Veillonella population than the 0.5 per cent lot (Fig. 4, *A*).

Stained smears were prepared from saliva samples during incubation. The population changes as shown above were not clearly apparent in bacterial smear

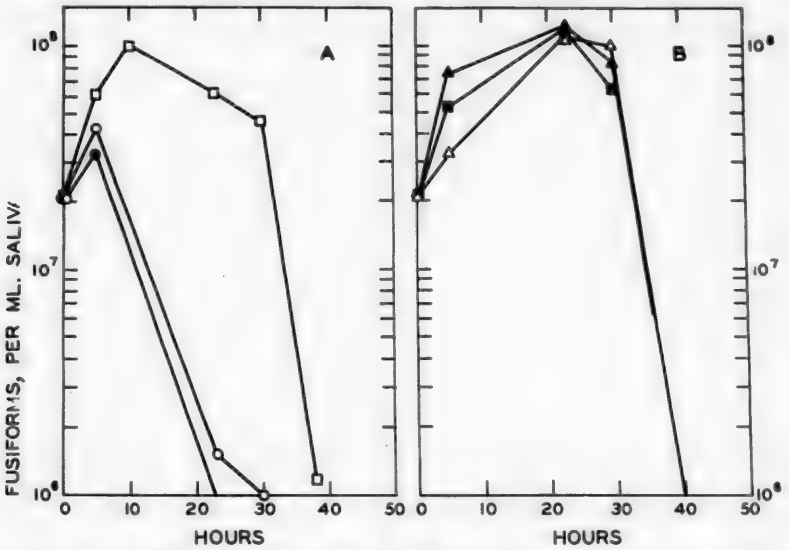

Fig. 2.—*A* and *B*, Effects of glucose, sodium lactate, and pH on fusiform populations in incubated saliva. (Legends are the same as in Fig. 1.)

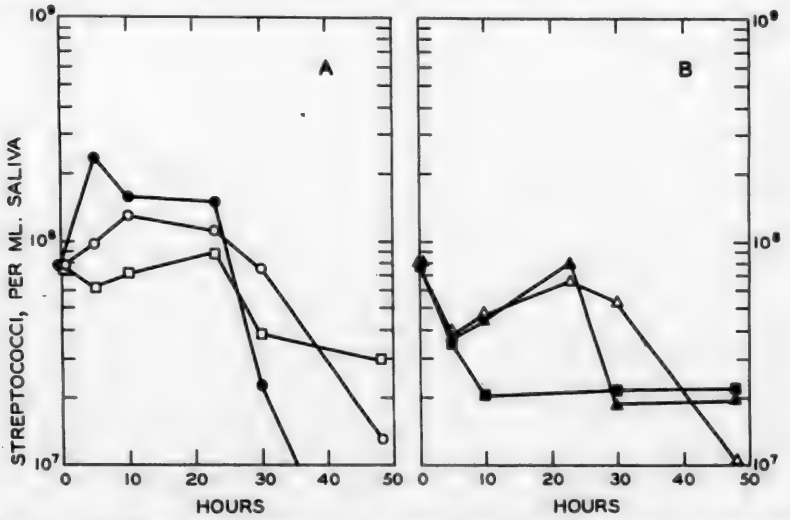

Fig. 3.—*A* and *B*, Effects of glucose, sodium lactate, and pH on streptococcal populations in incubated saliva. (Legends are the same as in Fig. 1.)

preparations which showed an adundance of several additional types of microorganisms such as spirochetes, diphtheroids, large and small cocci in chains or clusters.

The pH changes were followed in all saliva samples and lots (Table I). The results of preliminary experiments, reported earlier, correspond to Experiments 1 and 2. In the first experiment the pH increased from 7.55 to 7.70 at zero time to 8.00 to 8.20 at 30 hours. In the next experiment, the pH dropped upon incubation in all lots. The greatest drop occurred when glucose was

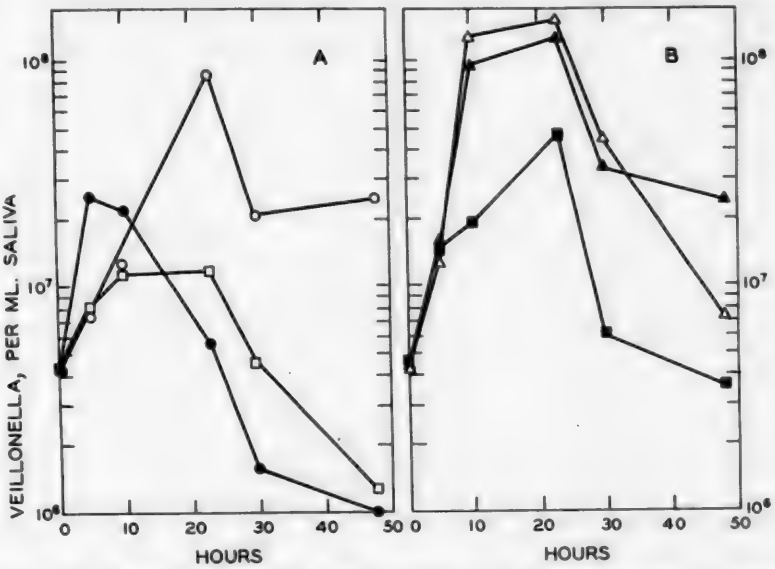

Fig. 4.—*A* and *B*, Effects of glucose, sodium lactate, and pH on Veillonella populations in incubated saliva. (Legends are the same as in Fig. 1.)

TABLE I
pH CHANGES IN INCUBATED SALIVAS

EXPERIMENT	SAMPLE	pH VALUES AT HOURS INDICATED				
		0	6.5	10	24	30
1	X	7.55	7.55	7.56	8.10	8.20
	Y1	7.60	7.45	7.69	7.80	8.10
	Y2	7.70	7.64	7.55	7.64	8.00

	SALIVA LOTS	pH VALUES AT HOURS INDICATED						
		0	5	10	23	30	47	54
2	1. 0.2% glucose	7.43	5.90	5.20	5.77	5.80	6.03	6.10
	2. pH 7.2	7.20	6.80	6.80	6.85	6.85	6.90	7.09
	3. unaltered	7.6	7.05	7.05	7.20	7.10	7.20	7.20

	SALIVA LOTS	pH VALUES AT HOURS INDICATED					
		0	5	10	24	30	48
3	1. 0.2% glucose	7.70	5.30	4.80	4.50	5.00	5.20
	2. 0.5% glucose	7.80	5.10	4.60	4.10	4.20	3.90
	3. 0.2% Na lactate	7.85	7.10	7.20	6.90	7.00	7.30
	4. 0.5% Na lactate	7.80	7.10	7.00	6.50	6.60	6.90
	5. pH 6.5	6.50	7.50	7.20	7.00	7.20	7.30
	6. unaltered	7.70	7.37	7.40	7.30	7.40	7.50

added. The pH changes in the third experiment were similar to those in the second experiment. Here again, saliva with glucose added showed the most pronounced drop in pH.

The formation of ammonia and indole was also followed as a measure of microbial action. Fig. 5 shows the effects of glucose, sodium lactate, and pH on the indole production. Indole was formed most rapidly and to the greatest extent in saliva which was not altered. The rate and extent of indole formation was decreased by the addition of lactate and the lowered pH and completely inhibited by glucose. In preliminary experiments, however, 0.2 per cent glucose concentration decreased rather than completely inhibited this reaction.

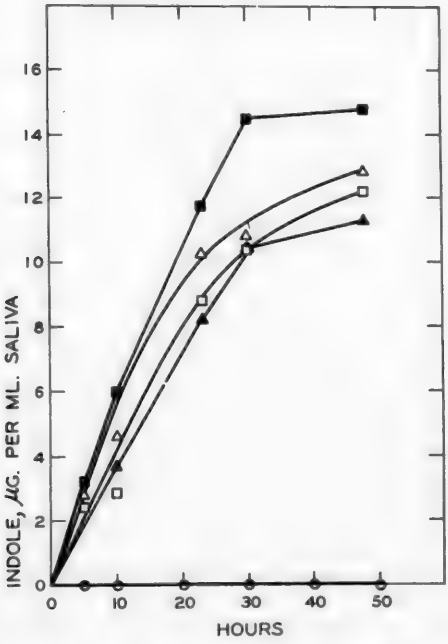

Fig. 5.—Effects of glucose, sodium lactate, and pH on indole formation in incubated saliva. (Legends are the same as in Fig. 1.)

The results obtained on the formation of ammonia (Fig. 6) parallel those of indole formation. The rate and extent of ammonia produced was greatest in the unaltered saliva. The addition of glucose caused a marked decrease in ammonia formation. Lactate at the 0.5 per cent level appeared to have decreased the amount of ammonia formed.

Total nitrogen and carbohydrate, as well as dry weight determinations, were carried out in order to evaluate the efficacy of these saliva samples to support bacterial growth. The results of these determinations are tabulated in Table II. By way of comparison, the carbohydrate and nitrogen content in saliva is lower than that found in the laboratory culture media. Although

saliva did support growth of bacteria, there was no apparent relationship between the glucose, nitrogen, and dry weight values and the bacterial population or chemical changes in the saliva studied.

TABLE II
NITROGEN, GLUCOSE, AND DRY WEIGHT VALUES OF SALIVAS

EXPERIMENT	SALIVA SAMPLE	TOTAL NITROGEN (μG/ML.)	FREE NH_3 NITROGEN (μG/ML.)	CARBOHYDRATE AS GLUCOSE (μG/ML.)	DRY WEIGHT (MG./ML.)
1	X	684	83	180	6
	Y1	481	99	170	6
	Y2	404	52	226	7
2	Pool	713	108	187	6
3	Pool	700	125	215	7

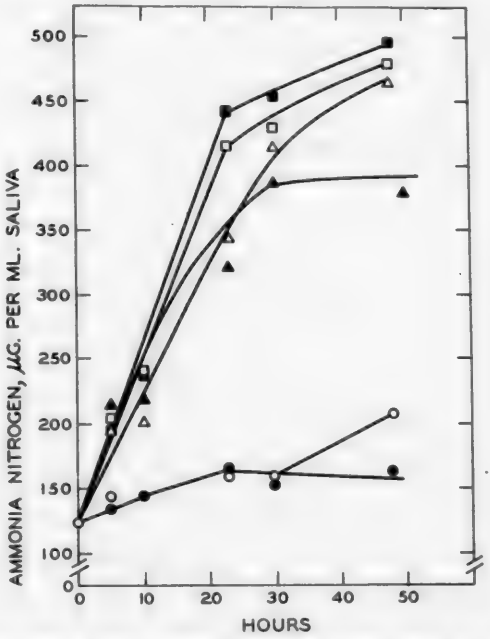

Fig. 6.—Effects of glucose, sodium lactate, and pH on ammonia formation in incubated saliva. (Legends are the same as in Fig. 1.)

DISCUSSION

It is an accepted fact that when whole saliva is incubated, the putrefactive and fermentative processes which take place are brought about by microorganisms. Nevertheless, extensive basic studies on saliva, such as those made on milk fermentation or sewage treatment, have been long overdue. The multitude of microbial processes, taking place in such a complex medium as saliva, is subject to change due to environmental factors. Hence, it was shown that the unaltered whole saliva, when incubated, supported the growth of at least the fusiform bacteria. However, the addition of glucose brought about the rapid death

of the fusiforms and a rapid growth of lactobacilli and, to a lesser extent, the streptococci. These changes are, by necessity, a small part of a still greater dynamic universe. A result of glucose fermentation is the lowering of pH. The lower pH could, in itself, favor the survival of the acidogenic bacteria as shown by the survival of the streptococci in the pH 6.5 saliva. The pH effect is illustrated by another example where neutral (or higher) pH favors the growth of certain bacteria. For example, the Veillonella appeared to be favored by a higher pH (pH 7.7), which is in agreement with the findings of M. Rogosa (private communication). Furthermore, the effects of glucose on the Veillonella population could be the result of (a) the lactate formed which brings about the initial rapid growth and (b) the acid formed which subsequently brings about the rapid death.

The inhibition of ammonia and indole formation by glucose in certain bacterial cultures has been known for some time. This inhibition has also been shown in incubated salivas by other investigators.[1, 11, 12] The formation of these end products by fusiforms is suggested by the proliferation of these microorganisms in stagnating saliva. Furthermore, the indole formation is characteristic of certain fusiform strains[13] and certain other bacteria. It is recognized that ammonia and indole formation can be brought about by bacteria other than those studied here and that these products can be formed by resting cells.

SUMMARY

Bacterial and chemical changes in incubated saliva were studied. The saliva studied contained 170 to 226 μg carbohydrate as glucose, 404 to 713 μg total nitrogen, and 52 to 125 μg free ammonia nitrogen per milliliter, prior to incubation.

During incubation, the formation of ammonia and indole were markedly inhibited by the addition of glucose.

Lactobacillus and streptococcal populations were increased, or maintained, at a higher level in the presence of glucose. Fusiforms, on the other hand, grew and maintained themselves in the absence of glucose. Veillonella increased in numbers in saliva with added sodium lactate and to a lesser extent with added glucose.

The authors are indebted to Dr. Ralph Lloyd for his cooperation in obtaining saliva specimens.

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THE GLYOXYLIC REACTANT IN SALIVA

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FOR some years the idea has been recurrent in dental literature that the salivary level of tryptophane or a similar indole-containing molecule may bear some relationship to dental decay. This was first pointed out by Turner and Crowell,[1] using the glyoxylic-sulfuric test for tryptophane, and it was indicated that tryptamine might be involved. The association with dental decay was confirmed by Green, Dodd, and Radik,[2] using similar reagents. However, Stack,[3] using dimethylaminobenzaldehyde as a measure for tryptophane, asserted the independence of salivary tryptophane and caries experience. Turner and Anders[4] studied the dimethylaminobenzaldehyde method with various indole derivatives and found that this test is essentially non-differentiating for tryptophane and indole-3-acetic acid. Among the amino acids the glyoxylic-sulfuric test is specific for tryptophane, but there are various indole containing substances which react to this test. This study was carried out to aid in interpreting the meaning of this test in saliva.

GENERAL METHOD

In the tests reported below the following modification of the Hopkins and Cole technic for the estimation of tryptophane was used:

To 1 ml. of the solution to be tested was added 1 ml. glyoxylic acid (pH 4.5). After mixing, 2 ml. concentrated sulfuric acid was dropped cautiously through the mixture. The sample was then allowed to stand for 10 min. in order to develop the maximum color. A Beckman model B spectrophotometer was used to read the optical density of the color. The reference standard was prepared of 1 ml. distilled water, 1 ml. glyoxylic acid, and 2 ml. sulfuric acid. When this method is applied to saliva, we have called the unit or units which develop color in the test, the *glyoxylic reactant in saliva.*

The following report describes (I) a study of the glyoxylic reactant(s) in saliva, and (II) a comparison of these reactants with pure substances.

I. THE GLYOXYLIC REACTANT IN SALIVA

Method and Material.—The glyoxylic-sulfuric test was first applied to the supernatant of centrifuged saliva from children, and the optical density of the color against the reference standard was read in the spectrophotometer at 570

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mμ. The children were from a group of the patients coming to the Forsyth Dental Infirmary for Children, Boston, Mass., for regular treatment. The total number of children examined was 192; the ages ranged from 4 to 15 years. The saliva was collected without artificial stimulation in the morning, at least 2 hours after breakfast. The children were classified according to dental decay as caries-free and caries-susceptible individuals. Furthermore, in order to distinguish the extent of caries among those caries susceptible, the caries activity in the 12 months preceding the testing was calculated as a per cent, the number of new carious lesions being divided by the number of surfaces available to decay. Examination and diagnosis was done with mirror and explorer by the same person. The use of bite-wing radiographs in premolar and molar areas

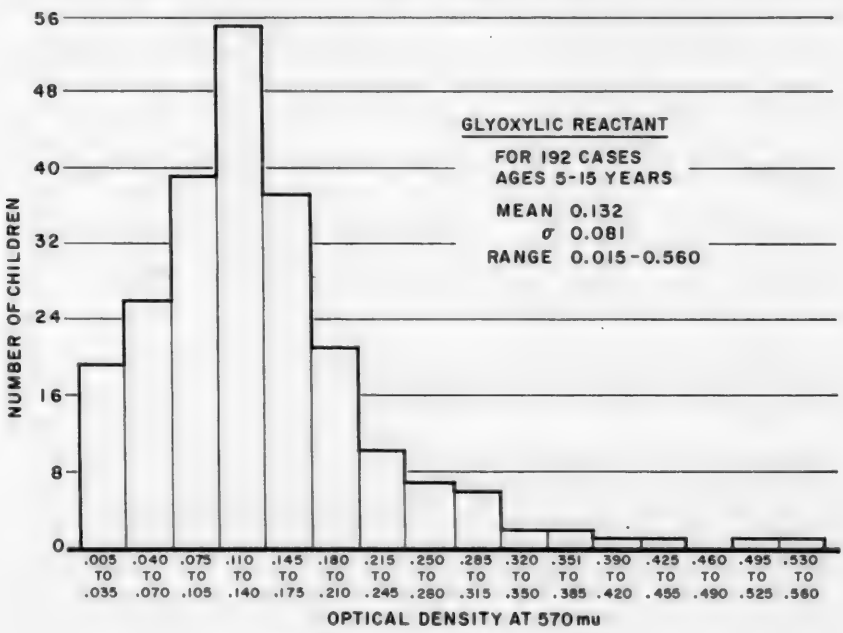

Fig. 1.—Frequency distribution.

aided in the diagnosis. The caries-free children had been followed over a 3-year period. They were at the time of testing, and had been during the period of observation, free from dental decay by mirror and explorer examination, and bite-wing radiographs taken at periods of 6 months. The caries extensive had also been followed over a period of time. Their dental decay was extensive, active, and rapid. The figure for per cent caries activity was derived by dividing the number of surfaces involved in decay during the past 12 months by the number of surfaces available for decay.

Results.—The frequency distribution of the glyoxylic reactant in the supernatant of the saliva from all 192 children is shown in Fig. 1.

In this group there were 9 caries-free children (Group 1). In order to compare these with a group having the most extensive dental decay, 9 of the caries susceptible, who had the highest per cent activity in the past 12 months, were selected according to matching age (Group 2). As previous findings[5] had shown that children with arrested decay in some of the teeth had a 55 per cent higher level of the glyoxylic reactant than other children of the group of whom they were a part, all children with arrested decay were excluded from the extensive group. The results are shown in Table I. All children with a caries activity of 8.8 per cent and over had a mean glyoxylic reactant of 0.108.

TABLE I

COMPARISON OF INDIVIDUAL CHILDREN IN GROUPS 1 AND 2

GROUP 1 : CARIES FREE		GROUP 2 : CARIES EXTENSIVE		
AGE (YR.-MO.)	GLYOXYLIC REACTANT (OPTICAL DENSITY, ABS.)	AGE (YR.-MO.)	GLYOXYLIC REACTANT (OPTICAL DENSITY, ABS.)	CARIES ACTIVITY (PER CENT)
7-6	0.275	7-7	0.045	10.7
7-10	0.105	7-8	0.100	8.8
11-1	0.155	11-6	0.130	10.0
11-5	0.135	11-10	0.060	11.8
12-9	0.200	12-7	0.150	10.1
13-0	0.135	12-11	0.020	10.8
13-2	0.150	13-4	0.185	17.6
14-0	0.120	14-0	0.115	16.0
14-5	0.190	14-4	0.055	25.6
mean = 0.163 $\Sigma = 0.049$ n = 9 range = 0.105-0.275		mean = 0.096 $\Sigma = 0.051$ n = 9 range = 0.020-0.185		

The difference between Group 1 and Group 2 is significant when the "t" test is applied. The confidence level is greater than 98 per cent.
"t" = 2.79.

When only wide extremes with respect to dental decay are considered, there is an indication that smaller amounts of the glyoxylic reactant, read at 570 mμ, tend to be found in the saliva of persons with extensive, active, rapid dental decay, while larger amounts of the glyoxylic reactant tend to be found in the saliva from caries-free persons. However, the glyoxylic reactant levels as read at 570 mμ are not systematically related to varying degrees of dental caries experience. The relatively large value of Σ in Group 2 might be indicative of interfering units.

Discussion.—Among the caries-extensive children, there were a few whose saliva showed a large amount of the glyoxylic reactant; the color of these samples was commonly deep cherry-apricot, in contrast with the usual color of lavender-blue, or purple. The samples were never colorless. The lowest optical densities, mostly found in the caries-extensive group, had a yellow or apricot color, apparently without any lavender-blue color.

This indicates that it is not tryptophane alone which yields a color with the glyoxylic-sulfuric test in saliva, and that other molecules may be interfering.*

*As this paper was being sent to press the very interesting and stimulating article of Edmonds, Bell, and Beerstecher[6] appeared. It is of considerable interest to note the measurement of *free* tryptophane in saliva by microbiologic assay with *Leuconostoc mesenteroides* yields different results with respect to dental decay, than does the glyoxylic acid reactant which does not differentiate free and combined tryptophane nor tryptophane from tryptamine. Consistency of salivary tryptophane levels was strongly noted by Edmonds and co-workers.

In order to investigate this problem further spectrophotometric analysis of the glyoxylic reaction products of salivas were compared with those of pure indole-containing substances.

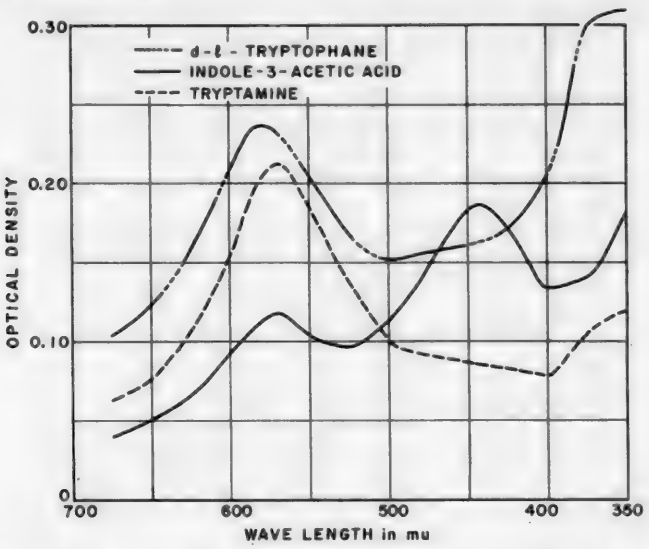

Fig. 2.—Absorption spectra for the glyoxylic reaction products of pure chemical substances.

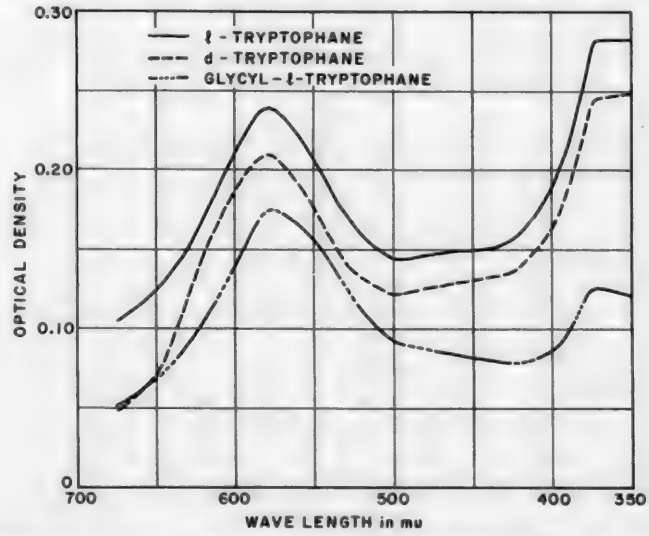

Fig. 3.—Absorption spectra for the glyoxylic reaction products of pure chemical substances.

II. COMPARATIVE STUDY OF THE GLYOXYLIC REACTANT IN SALIVA AND PURE INDOLE-CONTAINING SUBSTANCES

Method and Material.—The glyoxylic-sulfuric test as described above was applied to the solutions to be tested. The optical density of the glyoxylic reaction

products was read in the spectrophotometer at wave lengths ranging from 675 mμ to 350 mμ. The same reference standard as above was used. The glyoxylic-sulfuric test was first applied to known chemical substances. These were made up as 0.1 per cent solutions in distilled water, and further diluted in order to get comparable optical densities in the spectrophotometer. Comparisons were made with the results of the glyoxylic-sulfuric test applied to centrifuged saliva and to various mixtures of known chemical substances.

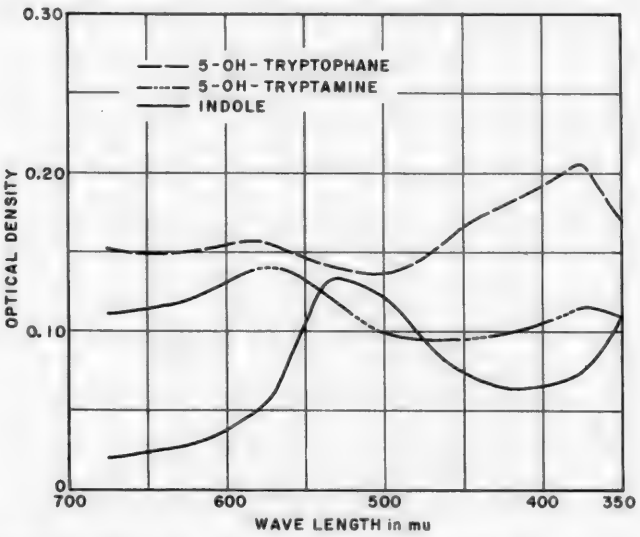

Fig. 4.—Absorption spectra for the glyoxylic reaction products of pure chemical substances.

TABLE II

COLOR AND ABSORPTION SPECTRA OF THE GLYOXYLIC REACTION PRODUCTS OF PURE CHEMICAL SUBSTANCES

% SOL.	SUBSTANCE	COLOR	PEAKS OF ABSORPTION SPECTRA
0.01	d-tryptophane	blue with black	narrow, 580 mμ
0.01	l-tryptophane	blue with black	narrow, 580 mμ
0.01	dl-tryptophane	blue with black	narrow, 580 mμ
0.0033	glycyl-l-tryptophane	lavender-blue	narrow, 575 mμ
0.0067	5-OH-tryptophane	bluish-green	plateau, 590-580 mμ; second peak, 375 mμ
0.0033	tryptamine-HCl	lavender-blue	narrow, 570 mμ
0.05	5-OH-tryptamine	blue	plateau, 575-570 mμ; second peak, 375 mμ
0.001	indole	cherry-red	narrow, 530 mμ
0.005	indole-3-acetic acid	greenish-yellow	low, broad, 570 mμ; high, narrow, 445 mμ

Results.—The absorption spectra of the single known chemical substances are shown in Figs. 2, 3, and 4, and described in Table II.

For comparison the glyoxylic-sulfuric test was applied to the supernatant of centrifuged saliva from 16 children. The color was noted and the results

of the optical density at different wave lengths were averaged according to the color of the glyoxylic reaction products when tested. The absorption spectra of the six visually different colors are shown in Figs. 5 and 6.

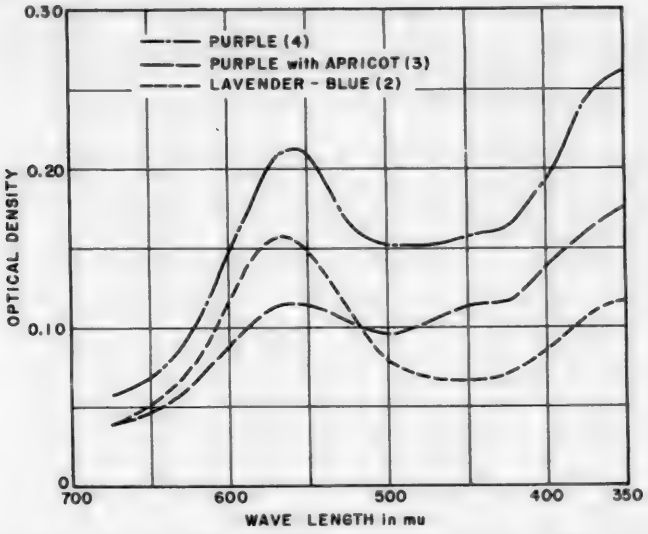

Fig. 5.—Absorption spectra of the glyoxylic reaction products in salivas (averaged according to color).

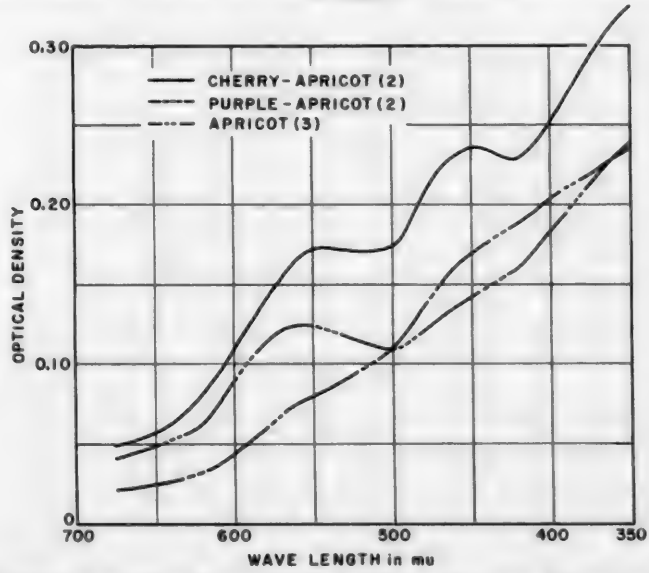

Fig. 6.—Absorption spectra of the glyoxylic reaction products in salivas (averaged according to color).

Fig. 5 shows 3 colors:

1. Lavender-blue color (2 salivas): Narrow peak at 570-565 mμ, lowest point at a lower wave length is at 450 mμ. The optical density at the peak is more than two times the optical density at 450 mμ.

2. Purple color (4 salivas): Narrow peak at 555 mμ, slight elevation at 450 mμ. The optical density at the peak is ⅓ higher than the optical density at 450 mμ.

3. Purple with apricot color (3 salivas): Broad peak at 565-560 mμ, a suggestion of a peak at 450 mμ. The optical density at the peak at 565-560 mμ is the same as the optical density at 450 mμ.

Fig. 6 shows 3 colors:

1. Purple-apricot color (2 salivas): Broad peak at 555 mμ, lowest point at a lower wave length is at 500 mμ. The optical density at 450 mμ is a little larger than the optical density at the peak.

2. Cherry-apricot color (2 salivas): Peaks at 545 mμ and 450 mμ. The optical density at the peak at 545 mμ is ¾ of the optical density of the peak at 450 mμ.

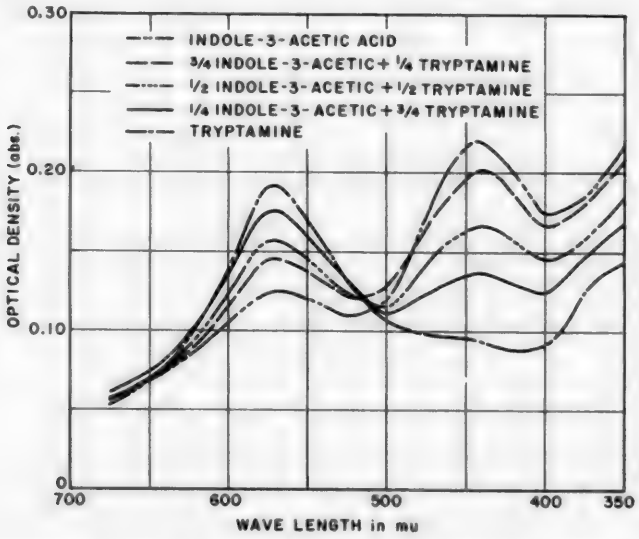

Fig. 7.—Absorption spectra of the glyoxylic reaction products of mixtures of tryptamine and indole-3-acetic acid.

3. Apricot color (3 salivas): Nearly a straight line. The optical density is steadily increasing from 675-350 mμ. Very small suggestions of peaks in the regions of 560 and 450 mμ.

In order to study further the chemistry related to the different spectra and colors of the glyoxylic reaction products of salivas, the following chemical substances were mixed in different proportions, and the glyoxylic-sulfuric test was applied:

tryptamine-HCl (0.0025%) and indole-3-acetic acid (0.005%)
tryptamine-HCl (0.0025%) and indole (0.0005%)
indole (0.0005%) and indole-3-acetic acid (0.005%)
tryptamine-HCl (0.0025%), indole (0.0005%) and indole-3-acetic acid (0.005%)

The absorption spectra are shown in Figs. 7, 8, 9 and 10.

Discussion.—In this attempt to throw some light on the nature of the glyoxylic reactant in different salivas, we have seen that:

The lavender-blue color in the samples and their absorption spectra resemble the glyoxylic reaction products of tryptamine and glycyl-l-tryptophane as well as tryptophane.

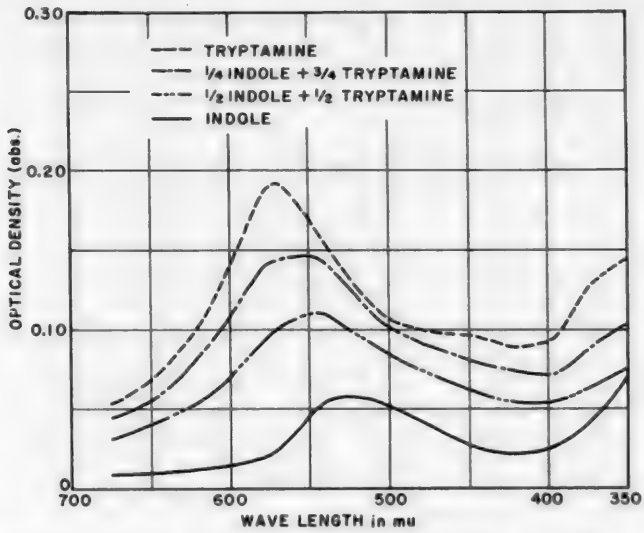

Fig. 8.—Absorption spectra of the glyoxylic reaction products of mixtures of tryptamine and indole.

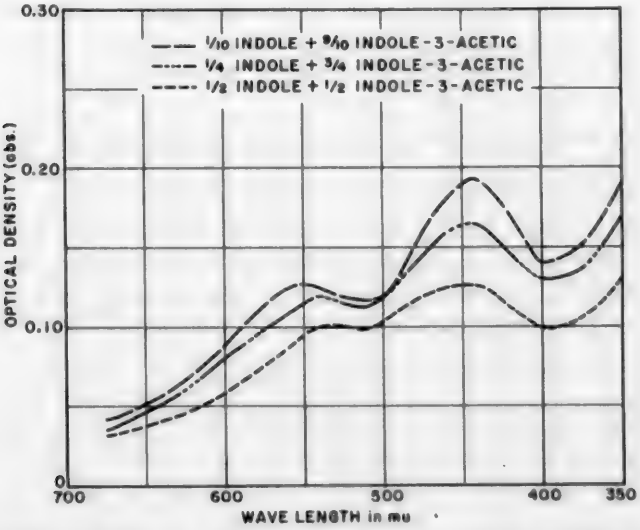

Fig. 9.—Absorption spectra of the glyoxylic reaction products of mixtures of indole-3-acetic acid.

The shift of the peak at 570 mμ for the glyoxylic reaction products of tryptamine gradually toward 545 mμ, in the purple, purple with apricot, and purple-apricot saliva samples, might be due to small amounts of indole.

The definite peak at 450 mμ in the cherry-apricot saliva samples, and the attempt to a peak at 450 mμ in the purple, and purple with apricot samples, is probably due to indole-3-acetic acid.

However, we are not able to account for the strange absorption spectrum in the apricot samples, and in the purple-apricot samples below 500 mμ. Other molecules may cause this interference,* and it could very well be due to carbohydrate browning.

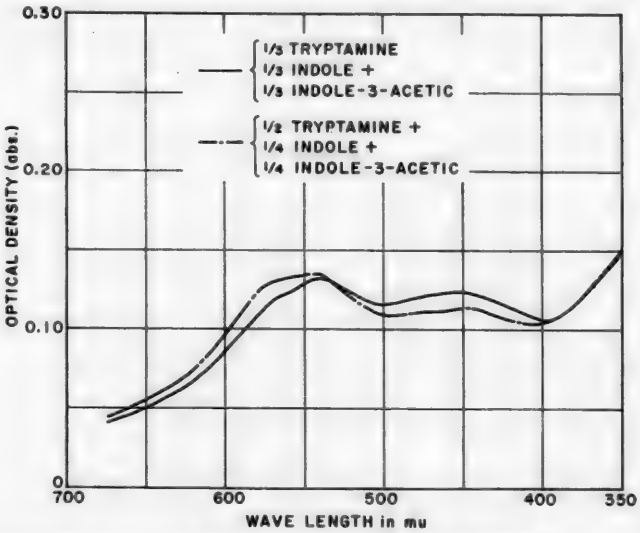

Fig. 10.—Absorption spectra of the glyoxylic reaction products of mixtures of tryptamine, indole, and indole-3-acetic acid.

It should be mentioned that these glyoxylic reactants in saliva are probably linked together or to other molecules, since much reactant remained in a dialysis sac under mercury pressure over a 60-hour period.

A selective solvent technic may be desirable in order to define more clearly the units involved here. To measure differentially free and combined tryptophane (or similar indole containing molecule) may be a clarifying step. Paper chromatography on hydrolysates of saliva might yield results of interest.

SUMMARY AND CONCLUSIONS

A comparative, spectrophotometric study of the glyoxylic reactant in saliva on one hand and tryptophane and other indole-containing molecules on the other is presented.

*Green, Dodd, and Radike[2] decreased the concentration of sulfuric acid to avoid undesirable reactions with other constituents of saliva.

A good estimation of tryptophane and tryptamine is attained at 570 mμ, but indole and certain other molecules probably interfere with complete clarity of interpretation. To follow the spectrophotometric pattern at different wave lengths might be more meaningful than simply to read absorbance at 570 mμ.

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FILM THICKNESS AS A LIMITING FACTOR ON THE ABILITY OF VARIOUS CHEMICALS TO INHIBIT GLYCOLYSIS IN SALIVARY SEDIMENT

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THERE have been numerous studies concerning the ability of chemicals to inhibit acid formation by oral microorganisms, using as test substrates, saliva-carbohydrate mixtures,[1-14] salivary sediment,[15] and dental plaques.[16-20] Only a few investigators have experimented with the problem of rate of permeation of chemicals into plaquelike films.[15, 16, 20, 22] Previous studies in this laboratory have shown that chemicals which are able to produce considerable inhibition of glycolysis, according to trials in saliva-glucose mixtures, either are inactive or require much higher concentrations for inhibitory action on thin films of salivary sediment.[21] Muntz and Miller proved that penetration of one compound in dental plaque material is strongly influenced by thickness of the plaque layer.[22] Samples of plaque material were homogenized and reconcentrated into layers of varying thickness by centrifugation. The inhibitory action of Zephiran in different concentrations was tested on 0.5, 0.75, and 1.0 mm. layers and was found to be strongly affected by the thickness of the layer.

In the course of evaluating activity of numerous chemicals obtained by a screening program, it was decided that the extent of influence of thickness on inhibitory action should be investigated. This presentation is concerned with tests on 52 chemicals, the majority of which are inhibitory on thin layers of salivary sediment.

EXPERIMENTAL METHODS

Chemicals for test were selected from a group of more than 200 which had been proved active by duplicate tests in a prior investigation involving a total of 3,000 compounds. The tests were performed by immersing salivary sediment for 20 minutes in a solution of the chemical. The thickness of sediment layer was controlled in the original screening, but was not accurately measured.

The plan of the current investigation was to carry out duplicate tests at 0.2 mm. thickness of sediment, either 1 or 2 tests at 0.5 mm., and a single test at 0.9 mm. Sediment was obtained from pooled saliva of volunteers who had previously been shown to produce considerable quantities of acid to produce a

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pH differential of 1.1 units or more. Details of methods of investigation have been published,[15] and an adaptation of that technic for preparing standard thicknesses of sediment has been presented.[23] Thicknesses of 0.2, 0.5, and 0.9 mm. were selected because they were the maximum range which could be handled readily with existing technics, and they also probably represent the range of thickness of dental plaques to be found in vivo.

Many of the active chemicals were soluble in water with difficulty and gave evidence of having their action limited because of inadequate solubility. Ten per cent aqueous propylene glycol was originally employed to increase solubility, and for this investigation higher quantities of propylene glycol were also tried. Chemicals were dissolved in 20 or 40 per cent aqueous propylene glycol, whichever was necessary to produce clear solutions. Just before testing, each solution of chemical was diluted 1 to 1 with a solution containing buffer, sugar, and supernatant from saliva. This produced solutions for testing containing 1 per cent test chemical, 0.01 M $NaHCO_3$-CO_2 buffer, 0.2 M glucose and 4.5 per cent supernatant from saliva; the propylene glycol concentration was either 10 or 20 per cent.

Pooled salivary sediment, obtained from several donors was pipetted out into thimbles, using 10 μl of sediment for each 0.1 mm. of sediment. The thimbles were pulled onto glass electrodes and adjusted to produce the required thickness of sediment layer,[23] then immersed in control solution for about 1 hour. Each test chemical was placed in contact with thimbles for 50 minutes, then it was withdrawn and replaced by control solution for a period of an hour to permit attainment of a new steady state. The first control solution was used as a rinse for a period of 1 minute. Activity was calculated by dividing the second control pH differential by the first control differential. Under these circumstances complete inhibition would indicate a zero recovery and lack of inhibition would produce recovery values approximating 100 per cent.

The action of 10, 20, and 30 per cent propylene glycol on sediments of different thicknesses is presented in Table I. Figures in parentheses show numbers of tests averaged. The results indicate 10 and 20 per cent propylene glycol has a consistent and minimal influence on sediment activity, whereas the higher concentration generally produces inhibition. For this reason all tests herein reported are limited to chemicals tried in solutions containing 20 per cent propylene glycol or less.

TABLE I
EFFECT ON GLYCOLYSIS OF DIFFERENT CONCENTRATIONS OF PROPYLENE GLYCOL AT VARIOUS SEDIMENT THICKNESSES
(VALUES ARE RECOVERY IN PER CENT)

CONCENTRATION OF PROPYLENE GLYCOL IN WATER SOLUTION	THICKNESS OF SEDIMENT					
	0.2 MM.		0.5 MM.		0.9 MM.	
	RANGE	AVG.	RANGE	AVG.	RANGE	AVG.
10	59- 90	84 (4)	93-103	98 (2)	88-118	103 (2)
20	77-104	88 (6)	87-113	104 (4)	92-126	109 (3)
30	53-116	74 (4)	57-121	79 (3)	60- 66	63 (2)

TABLE II

EFFECT OF 52 CHEMICALS ON GLYCOLYSIS OF VARIOUS THICKNESSES OF SALIVARY SEDIMENT (VALUES ARE RECOVERY OF GLYCOLYSIS WHERE CONTROL = 100)

NAME	CONCENTRATION OF PROPYLENE GLYCOL (%)	SEDIMENT THICKNESS 0.2 MM.	0.5 MM.	0.9 MM.
Chemicals With Less Than 21 Per Cent Recovery				
N-(2-aminoisobutyl) aniline	10	−10	58	53
3, 3′-diaminodipropylamine	10	15	55	100
Kerylbenzyltrimethyl ammonium chloride	20	17	36	84
Iodoacetamide	10	18	24	61
2-ethylhexylethylenediamine	10	−09	73	90
Average		6	49	78
Chemicals With 21-40 Per Cent Recovery				
Chloro-alpha-methyl benzyl ethylene diamine	10	22	47	123
Cresyl "Cellosolve"	20	24	40	51
5-di-n-butylaminomethyl-3, 5-dimethyl-2, 4-oxazolidinedione	20	27	45	71
Phenol, 4-allyl-2, 6-dimethoxy-	20	27	50	42
Lauryl trimethyl ammonium chloride	10	28	78	81
2-ethyl butanol	20	28	27	86
Sesamol	10	29	48	92
Di coco dimethyl ammonium chloride	10	29	47	96
1, 1-dichloro-1-nitropropane	20	30	63	57
Guaiacol	10	30	61	137
2-(dibutylamino)ethylphenyl ketone	25	30	38	97
Piperidine, 1-p-anisoyl-	10	30	35	93
Armeen 10D (decylamine)	20	34	62	65
di-(p-octylbenzyl dimethyl ammonium chloride)butyne-2	10	34	38	66
2-ethylhexyl "Carbitol"	20	34	50	50
1, β oxyethyl 2-undecylimidazoline	20	36	67	92
2-cyclohexene 1-caproic acid	10	38	42	92
Cyclohexyllactate	20	38	64	99
Average		30	50	83
Chemicals With 41 to 60 Per Cent Recovery				
N-(p-chlorodiphenylmethyl)-N′-methyl piperazine HCl	10	41	43	94
Rosin amine D hydrochloride	20	41	64	74
4-amino-2-methyl-3-hexanol	10	41	102	116
2,4-dichlorophenol	10	43	103	93
Gentian violet	10	43	60	104
3,3′diaminodipropylamine	20	44	77	84
Quinone	10	45	94	88
1,3-dichloro-5,5-hydantoin	10	45	80	111
1,2,3,6-tetrahydrobenzaldehyde	20	50	80	78
2-allylphenyl "Cellosolve"	10	52	91	110
1,3,6,8-tetrazatricyclo[6.2.1.1^{3,6}] dodecane	20	52	58	83
2,4,6-tri(dibutylaminomethyl) phenol	10	52	102	117
1-hydroxyethyl-2-glyoxalidinyl)2-ethyl hexoymethyl) methane	10	55	103	108
Alkyl (C_9-C_{18}) tolyl methyl trimethyl ammonium chloride	10	57	77	112
2-nitro-2-methyl-1-propanol	10	58	120	147
2-nitro-2-methyl-3-hexanol	20	58	63	36
B-ethoxy propionaldehyde	20	60	86	91
Average		49	83	97

TABLE II—CONT'D

NAME	CONCENTRATION OF PROPYLENE GLYCOL (%)	SEDIMENT THICKNESS 0.2 MM.	0.5 MM.	0.9 MM.
Chemicals With 61 to 80 Per Cent Recovery				
Glycine, N-(N-[alpha-bromopropionyl] glycyl)	10	63	130	160
Octadecenyl dimethyl ethyl ammonium bromide	20	64	100	96
Bicyclo(2,2,1)-5-heptene-2-methanol	10	65	85	104
Salicylaldehyde	10	68	78	95
Versene T	10	69	94	118
N-hexyl "Carbitol"	10	72	70	162
p-phenetidine	10	72	89	100
Triketohydrindene	20	76	88	105
1,3-diethylurea	20	76	73	87
2-cyclopentene-1-valeric acid	10	78	75	88
Diethyl sulfite	20	78	85	73
2-chloro-2-nitropropane	20	79	74	84
Triethanolamine	10	80	95	105
		72	87	106
Chemicals With 81+ Per Cent Recovery				
Allyl diglycol carbonate	10	82	75	96
Bicylclo(2,2,1)-5 heptene-3-methyl "Cellosolve"	10	86	144	140
Phenyl ethanol 2 amine	10	94	102	121
Paradiisobutyl cresoxy ethoxy ethyl dimethyl benzyl ammonium chloride	10	98	128	121
3-[2-(3,3,5-trimethylcyclohexyl) isopropoxy]-propanol	10	100	116	132
Thymol "Cellosolve"	10	103	112	141
2-ethylhexanediol-1,3	10	104	125	100
Lauryl pyridinium chloride	10	108	99	155
Benzyl alcohol	10	116	114	155
Average		99	113	129

The complete list of chemicals tested and the recoveries obtained at different thicknesses are presented in Table II. These chemicals are listed in order of increasing recovery values on sediments 0.2 mm. thick; that is, the first chemicals in the table have the greatest inhibitory action. In general, it can be observed that inhibition is greatest for thinner sediments and thickness profoundly affects the susceptibilty of the sediment to inhibitory action by nearly every chemical.

The general influence of thickness cannot be observed by taking averages of all tests, since the averaging of active and inactive chemicals may lead to erroneous conclusions. The chemicals were divided into 5 groups according to the order given in the table, consisting of ranges of 0-20 per cent, 21-40 per cent, 41-60 per cent, 61-80 per cent and over 81 per cent recovery. The means for each group were obtained and the data plotted in Fig. 1, with thickness on the horizontal axis and per cent recovery on the ordinate. All of these mean values fall upon a smooth curve, suggesting that inhibitory action is linearly related to the thickness of the sediment. The uppermost curve shows recoveries over 100 per cent for thicker films, which means that there is a

tendency for thick sediments to develop greater acid-forming capacity during the 3-hour test period, whereas the 0.2 mm. sediment does not do this. The tests in Table I on propylene glycol do not indicate as much stimulatory action at any of the three thicknesses of sediment. The uppermost curve in Fig. 1 lies 10 to 20 per cent above the control tests on propylene glycol, probably because different saliva donors were used in the 2 studies.

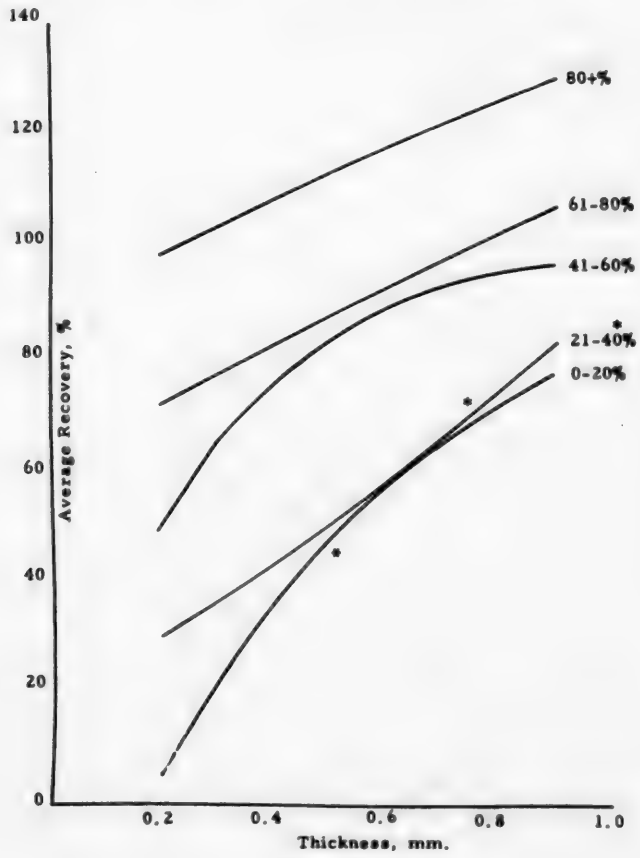

Fig. 1.

DISCUSSION

These findings constitute broad confirmation of the limited data of Muntz and Miller, that the thickness of dental plaque in vivo will probably effect the success with which inhibitory chemicals exert their action. The points indicated by asterisks on Fig. 1 are obtained from the findings of Muntz and Miller in their tests using reconstituted plaques with thicknesses of 0.5, 0.75, and 1.0 mm., and Zephiran at 1:1000 concentration. There is surprisingly good agreement between these findings on dental plaque and ours on salivary sediment.

The contact time selected, 50 minutes, may be representative of conditions that hold in case the chemical is administered via foodstuff, but it would certainly be too long to be representative of conditions which probably hold when inhibitor is used in dentifrice. Most of the chemicals will probably be less active when tested at periods of contact that are logical for representing dentifrice action. The ideal chemical for use in dentifrice should have different properties from the average of those tested. It should possess the ability to penetrate rapidly and to produce uniform inhibitory action regardless of the thickness of sediment over the range studied.

It is probable that the layers 0.2 mm. thick represent the types of plaques that are formed within a period of 24 hours over the gingival third of teeth, whereas the layers 0.9 mm. thick would represent the amount of heterogeneous material that germicides or inhibitor would have to penetrate in order to be effective in interproximal spaces or occlusal pits and fissures. These data also suggest that most chemicals should be much more effective in preventing Class 5 cavities than in preventing interproximal or occlusal cavities.

SUMMARY

Fifty-two chemicals, selected because of initial promise as glycolysis inhibitors, were tested for their inhibitory action on 3 different thicknesses of thin films of salivary sediment. Film thicknesses of 0.2, 0.5, and 0.9 mm. were prepared in contact with a glass electrode and supported by stiffened nylon mesh. After the pH drop had been measured in a control solution, the sediment was treated with a solution of the test chemical containing 20 per cent or less of propylene glycol as an accessory solvent. Next, the electrodes were transferred to the control solution for 1 hour to allow attainment of a new steady state. The percentage recovery was calculated from the ratio of the second to the first control pH differentials. The results were grouped according to the recoveries at 0.2 mm. thicknesses and averaged in 5 groups. Recovery was generally proportional to thickness. The most inhibitory group of chemicals were affected to the greatest extent by change in thickness. The groups of chemicals showing more than 40 per cent recovery on 0.2 mm. films possessed little or no inhibitory action when tested on 0.9 mm. films.

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THE INFLUENCE OF SIALOADENECTOMY IN RATS ON FOOD AND WATER CONSUMPTION

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SURGICAL removal of one or more pairs of the major salivary glands has been shown to cause major increases in the initiation and progression of carious lesions in the white rat.[1-8] In addition, sialoadenectomized rats grow more slowly than normal animals[5, 7, 8] and various changes have been reported in their adrenals, testes, and uteri.[9, 10] In view of these several suggestions of deranged metabolic circumstances in sialoadenectomized rats, the food and water consumption of normal and operated rats from a caries-resistant strain and a caries-susceptible strain were studied while they were being maintained on a cariogenic diet.

EXPERIMENTAL

Two experiments were conducted: the first with the Harvard caries-resistant strain of white rats that was continued for 90 days after surgery; the second with the mutant albino strain of moderately caries-susceptible white rats which was conducted over a 75-day period after surgery. The 2 experiments were otherwise identical in design. The mutant albino strain is not the same as the Harvard caries-susceptible strain, the offspring of which are used in most studies conducted in these laboratories. The mutant albino strain is being developed from different stock in order to have a second independent strain of caries-susceptible rats for experimental purposes. At present the rats in the latter strain have a greater variability and a somewhat lower caries-susceptibility than the rats from the Harvard caries-susceptible strain.

The subjects in both these experiments were born to females in the respective colonies that had been maintained throughout their entire lives on Harvard cariogenic ration No. 700.[11] After weaning at 21 days of age, the offspring were placed in individual, wire-bottom cages and offered ration No. 700 and tap water ad libitum. In both experiments, there were 4 groups of rats: intact males, intact females, sialoadenectomized males, and sialoadenectomized females. Littermates were distributed equally among the groups. The rats that were to be operated upon were allowed to stabilize until 30 to 32 days of age. Then the principal salivary glands (parotid, submaxillary, and major sublingual) were removed during ether anesthesia. Food consumption was recorded daily in both experiments. Water consumption was recorded in the second experiment only.

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TABLE I

THE INFLUENCE OF SIALOADENECTOMY IN WHITE RATS ON GROWTH, AND ON FOOD AND WATER CONSUMPTION

GROUP	NO. OF RATS	WEIGHT INCREASE			TOTAL FOOD CONSUMPTION			FOOD CONSUMPTION PER GRAM OF BODY WEIGHT INCREASE			TOTAL WATER CONSUMPTION		
		AVERAGE (GM.)	SEM* (GM.)	CR†	AVERAGE (GM.)	SEM* (GM.)	CR†	AVERAGE (GM.)	SEM* (GM.)	CR†	AVERAGE (ML.)	SEM* (ML.)	CR†
Experiment No. 1—Harvard Strain													
Intact ♂	5	312	17	8.1	1367	94	3.2	4.4	0.3	5.3	-	-	-
Intact ♀	17	167	7	4.8	1031	45	1.8	6.3	0.2	2.6	-	-	-
Sialo. ♂	5	224	7	4.0	1605	90	4.1	7.2	0.4	5.6	-	-	-
Sialo. ♀	15	123	8	9.2	1424	86	1.5	12.0	2.2	2.2	-	-	-
Experiment No. 2—Mutant Albino Strain													
Intact ♂	6	239	22	4.8	1271	62	3.3	5.6	0.6	2.5	2287	332	1.0
Intact ♀	7	129	8	2.6	1027	41	1.0	8.3	0.9	2.7	1917	163	1.8
Sialo. ♂	9	175	12	2.2	1357	55	1.8	8.1	0.7	2.3	3194	370	2.1
Sialo. ♀	6	103	8	5.1	1162	64	2.3	11.7	1.1	2.8	2458	204	1.7

*SEM—Standard error of the mean.

†CR—Critical ratio. The critical ratio is the ratio of the difference between two means to the standard error of the difference between the means. Wherever the critical ratio is less than 2.0, the difference between the means is considered to be statistically insignificant; when from 2.0 to 2.9, moderately significant; when 3.0 or higher, highly significant.

After the respective experimental periods, which had been determined on the basis of the time when gross carious lesions first could be observed in vivo in the rats in the sialoadenectomized groups, the rats were sacrificed and their heads evaluated for carious lesions by the procedure of Shaw, Schweigert, McIntire, Elvehjem, and Phillips.[12]

RESULTS

The data on the body weight increase, the total food consumption during the whole experimental period, the food consumption per gram of body weight increase and the total water consumption of the rats in these experiments are presented in Table I. In the first 3 of the above criteria, the results from the Harvard caries-resistant and the mutant albino caries-susceptible strains paralleled each other closely. No comparison between strains was possible with respect to water consumption.

Intact male rats grew much more rapidly than sialoadenectomized males; likewise normal female rats grew much more rapidly than sialoadenectomized females. The sialoadenectomized males appeared to be somewhat more seriously penalized with respect to increases in body weight than the sialoadenectomized females.

Despite a slower rate of growth, the sialoadenectomized males routinely consumed a greater amount of ration during the full experimental periods than their normal littermates; the same difference was noted for female rats. When the total food consumption was related to the increase in body weight during the experiments, the operated males and the operated females were found to be even more inefficient in their utilization of food than was evident from the data on total food consumption. In every case the food consumption per gram of body weight increase was significantly greater for the operated than for the unoperated rats. Normal and sialoadenectomized male rats routinely ate larger amounts of food during the experimental period than normal and sialoadenectomized female rats, respectively. However, the average food consumption per gram of body weight increase was significantly lower for male rats than for female rats in every comparison.

Water consumption by the operated rats was numerically higher than for intact rats. However, wide variations in water consumption were observed from rat to rat in both normal and sialoadenectomized groups. Hence the standard errors of the means were large, with the result that the differences in water consumption between groups, though numerically large, were of borderline statistical significance.

The data on the initiation and progression of carious lesions among the rats in these experiments are presented in Table II. The caries incidence among the intact Harvard caries-resistant male and female rats was in keeping with the general tendencies toward a high resistance to tooth decay observed in offspring of this strain. Likewise, the caries incidence among the intact mutant albino rats was in keeping with the higher caries-susceptibility of this strain. In both strains and in both sexes, removal of the principal salivary

TABLE II

THE INFLUENCE OF SIALOADENECTOMY IN A STRAIN OF CARIES-RESISTANT WHITE RATS AND IN A STRAIN OF CARIES-SUSCEPTIBLE WHITE RATS

GROUP	NO. OF RATS	NO. OF CARIOUS MOLARS			NO. OF CARIOUS LESIONS			EXTENT OF CARIOUS LESIONS		
		AVERAGE	SEM*	CR†	AVERAGE	SEM*	CR†	AVERAGE	SEM*	CR†
Experiment No. 1—Harvard Strain										
Intact ♂	5	4.0	1.5	0.2	6.2	3.2	0.4	12.2+	5.9+	0.4
Intact ♀	17	3.5	1.9	3.3	4.9	1.2	3.4	9.4+	2.8+	4.0
Sialo. ♂	5	9.8	0.9	3.4	18.2	2.5	9.3	57.2+	9.6+	8.6
Sialo. ♀	15	10.1	0.5	0.3	20.7	1.2	0.9	66.8+	6.1+	0.8
Experiment No. 2—Mutant Albino Strain										
Intact ♂	6	6.5	0.9	0.6	10.3	2.1	1.3	24.2+	8.5+	1.3
Intact ♀	7	7.3	0.9	3.4	14.0	2.0	4.6	38.9+	7.6+	5.1
Sialo. ♂	9	10.0	0.5	2.1	22.0	1.4	2.2	82.6+	7.7+	2.8
Sialo. ♀	6	9.8	0.8	0.2	20.3	0.7	0.7	71.8+	8.8+	0.9

*SEM—Standard error of the mean.
†CR—Critical ratio.

glands resulted in grossly visible, statistically significant increases in the numbers of carious molars, the number of carious lesions, and the extent of carious lesions. The greater caries-susceptibility of the mutant albino rats was still evident in the sialoadenectomized rats of this strain, as their caries incidence was appreciably greater than that of the Harvard sialoadenectomized rats, even though the latter subjects had been on experiment for an additional 15 days.

The data on dental caries incidence are not presented separately for the maxillary and mandibular molars. However, in both strains of rats, major increases in caries incidence were noted in the maxillary molars as well as in the mandibular molars of the sialoadenectomized subjects. As in the normal rats, the initiation and progression of carious lesions were routinely greater in the mandibular molars than in the maxillary molars.

DISCUSSION

These data present further information on the extent to which the removal of the major salivary glands penalized the young rat. Earlier studies have shown that sialoadenectomized rats grow less rapidly than normal ones of the same strain.[5, 7, 8] The same reduced rate of growth was observed among sialoadenectomized male and female rats of a caries-resistant strain and of a caries-susceptible strain in the present experiments. Bixler, Muhler, and Shafer[9] have reported the results of an experiment in which a paired-finding procedure was followed with the control rat being provided the amount of food eaten daily by its sialoadenectomized mate. With equal consumption of the natural diet used in this experiment, the operated rats still grew somewhat slower than the controls. In our studies where purified cariogenic diet No. 700 was used, the sialoadenectomized rats ate appreciably more than their control littermates. This observation is in direct contrast to the results of Bixler, Muhler, and Shafer where the sialoadenectomized rats ate less than their controls when both groups were fed ad libitum. In addition to the obvious difference in diet between the 2 experimental regimens, the operative procedure used in our laboratory may have been more severe as all fragments of the diffuse parotid gland were removed carefully. Support for the latter suggestion may be gained from the fact that the male and female rats in the sialoadenectomized groups of both of our strains grew more slowly than those reported by Bixler, Muhler, and Shafer. Although their experimental period was 140 days in contrast to 100 days for our caries-resistant strain and 85 days for our caries-susceptible strain, the control rats in both of our experiments had grown as much or more than the control rats in their unrestricted feeding experiment. Whereas our sialoadenectomized rats had been penalized from 41 to 46 per cent in body weight increase, the operated rats in Bixler, Muhler, and Shafer's study had grown 11 to 14 per cent less rapidly than their controls. Clearly some major difference exists between the operated rats in the 2 experimental regimens that would explain the difference in food consumption observed between the two investigations.

SUMMARY

The rate of growth of male and female sialoadenectomized rats was much less than that of their normal littermates.

The total food consumption and the food consumption per gram of body weight increase were greater for sialoadenectomized rats than for normal littermates.

The water consumption of sialoadenectomized rats from a caries-susceptible strain was greater than the water consumption of the control littermates.

Sialoadenectomy caused major increases in dental caries incidence in rats from a highly caries-resistant strain as well as in rats from a highly caries-susceptible strain. The rats from the caries-susceptible strain continued to demonstrate a higher caries-susceptibility after sialoadenectomy than operated rats from the caries-resistant strain. The increased caries initiation and progression was as evident in the maxillary molars as in the mandibular molars of both strains.

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EFFECT OF CERTAIN RESTORATIVE MATERIALS ON SOLUBILITY OF DENTIN

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A PREVIOUS paper reported the effects of several silicates, zinc phosphate cements, and restorative resins on the solubility of intact and powdered enamel.[1] Some of these materials did reduce the solubility, and this effect was associated with the presence of fluoride in the material. The possibility was suggested of imparting some anticariogenic properties to certain inert materials, such as resins, through this mechanism.

Although it has been established that enamel solubility is reduced by silicates and resins which contain fluorides, present knowledge concerning the lack of sealing properties of restorative materials suggests that the effect on dentin might also be important.[2-6] Thus, the purpose of this investigation was to study the effect of some of these materials on dentin solubility.

PROCEDURE

The commercial products purchased on the open market were:

Silicates

S. S. White Filling Porcelain	S. S. White Co.
de Treys Cement	L. D. Caulk Co.
Berylite	W. V. B. Ames Co.

Zinc Phosphates

S. S. White Zinc Cement	S. S. White Co.

Resins

Kadon	L. D. Caulk Co.
FluorOn	American Consolidated Co.

A number of experimental resin and zinc phosphate cements, containing 2 per cent and 5 per cent concentrations of certain fluoride compounds,* were also included in the investigation.

The procedures employed here were similar to those used in the enamel studies[1] and will only be briefly summarized.

A window-type test was used for studying intact surfaces. The labial enamel was removed by grinding on a wet stone to expose a flat area of dentin. This surface was polished by means of a felt wheel and Buhler's polishing alumina. The tooth was then entirely covered with a combination of

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*Prepared by the L. D. Caulk Co., Milford, Del.

electrical tape and wax, except for a circle, 3.5 mm. in diameter, of exposed dentin. The control or original solubility of each tooth was established by measuring the amount of calcium dissolved from this area during 30-minute decalcification in 10 ml. of pH 2.5 acetic acid. The tape and wax were subsequently removed and the tooth again buffed until all traces of the acid etch disappeared. The test material was then applied and after 15 minutes in a humidor, each tooth was placed in a test tube containing 15 ml. of distilled water. The storage time was 2 weeks with the water changed at 24-hour intervals. At the end of this period, the materials were removed and the surfaces lightly buffed to remove any debris or etching produced by the test material. The windows were then replaced to expose the same dentin area for the second solubility test. The effect of the materials was thus determined by noting the difference in the solubility of this region before and after exposure to the test materials.

In order to make sure that the observed differences in the original and subsequent dentin solubility were actually due to the materials themselves and not merely to normal variations in the dentin layer resulting from buffing or other experimental procedures, control tests were run. Eight teeth were prepared in the usual manner, decalcified and the solubility determined. The etched surfaces were then buffed but no test materials applied. The windows were simply replaced and the decalcification procedure repeated. The average variation in solubility was 3.3 per cent. There was no pattern of behavior with individual teeth, some exhibited slight increases and others slight decreases. The results were comparable to the observations when similar tests were conducted with enamel and indicate that the slight changes in depth as induced by the buffing procedure do not appreciably alter solubility.[7] There was, however, considerable variation in the behavior of individual teeth; hence the necessity of employing an adequate number of teeth with this type of test must again be emphasized. From 8 to 48 teeth were tested with each material.

All powdered dentin used in the investigation was of a particle size which would pass a 100-mesh but not a 140-mesh screen. It was separated from the enamel and cementum by a combination of the methods of Manly and Hodge[8] and Battistone and Burnett.[9] The solubility of the control (normal calcium solubility) was determined by measuring the solubility of 15 100-mg. samples taken from the dentin pool. Powdered specimens were decalcified for 20 minutes in 15 ml. of acetic acid, pH 2.5. At the end of this time, the acid was removed by vacuum filtration, and an aliquot analyzed for calcium. The mean quantity of calcium dissolved from these 15 samples was 2.368 mg. per cent with a standard deviation of 0.046 mg. per cent. This figure served as the control for all subsequent experiments with powdered dentin.

The treatment of the powdered specimens was accomplished by storing 130-mg. samples for 1 week in distilled water with discs (20 ± 1 mm. diam.)

of the materials. The water was syphoned off each day and replaced. At the end of 1 week, the solubility of 100-mg. samples was measured by decalcifying them in the same manner as the controls.

Glass-distilled water was used throughout and storage of specimens was done at 37° C. Mechanical agitation was employed with all decalcification procedures. Calcium was determined by the method of Solomon, Gabrio, and Smith.[10] Restorative materials were manipulated in accordance with the prescribed procedure for each particular material.

RESULTS

The effects of the various silicate cements on the intact dentin surfaces can be seen in Table I. A very marked reduction was noted in those surfaces exposed to S. S. White and de Treys' silicates. Both of these materials are prepared with a fluoride flux and both produced a similar reduction in enamel solubility. The dentin surfaces to which Berylite had been applied were also reduced in solubility, although not in the magnitude observed with the other two silicates tested. This reduction was rather surprising, however, since this material contains no fluoride and invariably increased the solubility of enamel surfaces which were exposed to it.

Since the behavior of S. S. White and de Treys' silicates was quite comparable on the intact surfaces, only S. S. White and Ames were used with powdered dentin. These average results can also be seen in Table I and tend to corroborate the data obtained in the intact surface tests. Again some reduction in solubility was noted with the Ames material.

TABLE I

EFFECTS OF VARIOUS SILICATE CEMENTS ON INTACT DENTIN SURFACES AND POWDERED DENTIN

SILICATE	AVERAGE PER CENT CHANGE IN CALCIUM SOLUBILITY	
	INTACT	POWDERED
S. S. White	-52.3	-34.8
de Treys	-58.2	
Ames Berylite	-30.2	-16.5

The effect on solubility of both intact and powdered dentin exposed to two commercial restorative resins and several experimental resins is seen in Table II. Kadon, a resin which contains no fluoride, had little effect on the solubility of dentin. When intact dentin was exposed to FluorOn, a commercial product containing unspecified quantities of both stannous and sodium fluorides, marked decreases in solubility were evidenced. The degree of reduction induced by the various experimental resins appeared to be somewhat dependent on concentration. The resins containing 5 per cent of the fluoride compounds generally reduced solubility more than those containing 2 per cent. No concentrations greater than 5 per cent were tested as it was felt that higher concentrations were beyond the realm of practicability. Concentrations below 2 per cent had offered little protection to enamel and for this reason were not included.

The effectiveness of some of the materials was also tested with powdered dentin. The relative effect was comparable to the data obtained with intact dentin.

TABLE II

EFFECTS OF VARIOUS EXPERIMENTAL AND COMMERCIAL RESINS ON INTACT DENTIN SURFACES AND POWDERED DENTIN

RESIN	AVERAGE PER CENT CHANGE IN CALCIUM SOLUBILITY	
	INTACT	POWDERED
Resin + 2% NaF	- 9.2	- 1.7
Resin + 5% NaF	-23.2	-17.4
Resin + 2% Na_2SiF_6	- 4.1	
Resin + 5% Na_2SiF_6	-18.0	
Resin + 2% ZnF	-15.5	
FluorOn	-63.1	-32.0
Kadon	- 4.8	+ 0.4

In Table III can be seen the changes observed in the solubility of surfaces after 2 weeks' contact with zinc phosphate cements. There was a slight increase in the solubility of surfaces which had been covered with S. S. White zinc phosphate cement. A similar increase had also been noted on intact enamel treated with zinc phosphate cement. The addition of fluorides, either calcium or sodium, to experimental batches had little effect in reducing the solubility. The extremely small differences noted in the solubility of surfaces exposed to the commercial brands and to experimental batches containing fluoride do not suggest a protective action. The data from powdered dentin experiments tend to support the data from the intact surface experiments.

Although there were some differences in the behavior of individual materials (Ames Berylite, for example) and in the actual magnitude of change, these data indicate that, generally, restorative materials affect the solubility of enamel and dentin in a similar manner.

TABLE III

EFFECTS OF VARIOUS COMMERCIAL AND EXPERIMENTAL ZINC PHOSPHATE CEMENTS ON INTACT DENTIN SURFACES AND POWDERED DENTIN

ZINC PHOSPHATE CEMENT	AVERAGE PER CENT CHANGE IN CALCIUM SOLUBILITY	
	INTACT	POWDERED
S. S. White	+6.6	-2.1
Zinc Phos. + 10% CaF_2	+2.8	+2.7
Zinc Phos. + 5% NaF	+5.4	
Zinc Phos. + CaF (liquid)	+2.0	-4.2
Zinc Phos. (No F-)		+8.7

These data, however, must not be construed as a recommendation by the authors of a material such as FluorOn, or that the experimental materials developed are suggested for clinical use. Factors such as the effect on the pulp must be adequately investigated.

SUMMARY

1. The solubility of dentin was reduced after contact with silicate cements. Those products which contained fluorine produced a greater reduction than those which did not.

2. Resin restorative materials to which fluorides were added reduced dentin solubility.

3. Zinc phosphate cements, even those to which fluorides were added, did not lower dentin solubility.

The authors wish to acknowledge the capable assistance of James Platt, Earl Ley, and Norman Chamberlain during the course of the investigation.

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THE EFFECT OF RESIDUAL STRESS AND WATER CHANGE ON THE DEFORMATION OF HYDROCOLLOID IMPRESSION MATERIALS

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A PREVIOUS report[1] from this laboratory was concerned with a quantitative measurement of water change in reversible hydrocolloid when stored in various media. The data indicated that the magnitude of the water change was, of course, dependent on the nature of the storage environment and the storage time. However, the fluctuation in water content when the impression was stored in 100 per cent humidity was slight, generally being only 0.1 to 0.2 per cent. The fact that distortion can readily be detected in a hydrocolloid impression when stored in 100 per cent humidity for only a short period of time leads to the speculation that the release of internal stress may actually be of equal importance to water fluctuation[2] in dimensional change.

Thus, the determination of the individual effect of these two variables, change in water content and release of internal stress, upon the dimensional stability of the impression was the purpose of this investigation. Several other variables, such as water fluctuation in alginate, were also investigated.

GENERAL PROCEDURE

Two popular commercial reversible hydrocolloids and 3 irreversible hydrocolloids, purchased on the open market, were used (Table I). Unless otherwise stated, the manufacturer's directions for the reversible hydrocolloids were carefully followed in regard to boiling time, storage temperature, tempering time and temperature and gelling period. For the alginates, the water-powder ratio, water temperature, and mixing time were all controlled according to the manufacturer's directions.

The same method for measuring water change was employed as in the previous study.[1] Briefly it consisted of filling a No. 000 Coor's crucible with hydrocolloid and using a weight-loss method to detect water change in these specimens. A temperature of 105° C. ± 5° was employed for drying, and the drying period was 48 hours. In some of the tests, it was necessary to measure the water content in actual dental impressions. In these cases, the entire impression was used, blotted dry, placed in a tared weighing bottle and the water determined.

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TABLE I

MATERIALS USED IN THIS INVESTIGATION

BRAND	BATCH NUMBER
Reversible Hydrocolloid	
Deelastic	nn 303-642, 661 nn—586
Van Rossem	5508192
Irreversible Hydrocolloid	
Jeltrate	23567
Coe-loid	C-11
D-P	2713

Two methods were employed for producing an increased residual stress pattern in the reversible hydrocolloid impressions. Since incomplete and rapid gelation is acknowledged as a possible common cause for inadequate gel strength, and increases the possibility of distortion,[3, 4, 5] one series of impressions was taken removing impressions at the end of 3 minutes after chilling with ice water. Another series was made by using the recommended 5-minute gelling time with circulating water at a temperature of 60° to 70° F. The other technic for increasing the stress was by use of undercut dies. It has been established that the greater the undercut, the greater is the elastic deformation and susceptibility to distortion. A National Bureau of Standards MOD steel die was placed and tilted in a stone base at varying angles. Using a protractor, angles of 10, 15, and 20 degrees were arbitrarily selected. As anticipated, the distortion was greatest with the die tilted at the 20-degree angle and, thus, this position was used in the succeeding tests as the technic for inducing stress. The control consisted of the steel die mounted vertically in the stone base. A 5-minute gelation time was used on these specimens.

The distortion of the impressions was measured by transfer of a snugly fitting gold casting from the master model to the hydrocal die. Thus, the casting served as the control, a technic used in other investigations.[3, 6] The distance, in millimeters, by which the casting failed to seat fully on the stone die under a 1-pound load was the value used. Measurements were made by use of a micrometer microscope. All of the dies were poured immediately from one stone, Vel-Mix,* at a constant water-powder ratio. The dies were allowed to set for a minimum of 24 hours before the casting was tried on. A minimum of 6 impressions was made for each series and the results shown are the mean values.

RESULTS

Water Change in Alginate.—The water fluctuation in 3 alginates stored in 3 media—air, distilled water, and approximately 100 per cent humidity—can be seen in Figs. 1-3. The results compare favorably with those which were obtained with reversible hydrocolloid. The greatest fluctuation occurred when specimens were stored in air while the least water change was in 100 per cent humidity. In fact, with 2 of the alginates the alteration in water content within the impression was remarkably minute. These data corroborate previous

*Kerr Mfg. Co., Detroit, Mich.

studies which indicate that syneresis and imbibition may be somewhat less with irreversible as compared to the reversible hydrocolloids.[7] However, the evidence is clear that dimensional change of clinical significance can be detected in

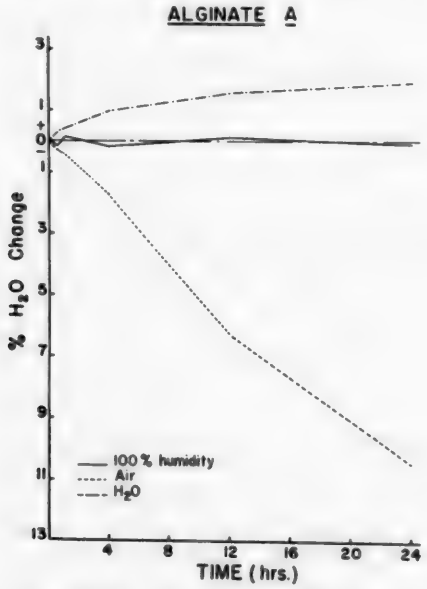

Fig. 1.—Water change in irreversible hydrocolloid A in various media.

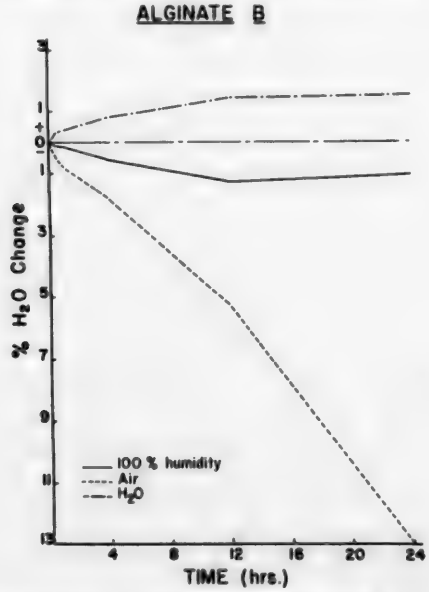

Fig. 2.—Water change in irreversible hydrocolloid B in various media.

alginate impressions within 24 hours, or less, even when stored in 100 per cent humidity. In view of the small water change in the impression, these observations again indicate that release of internal stress may be of greater, or at least of equal, importance in maintenance of dimensional stability.

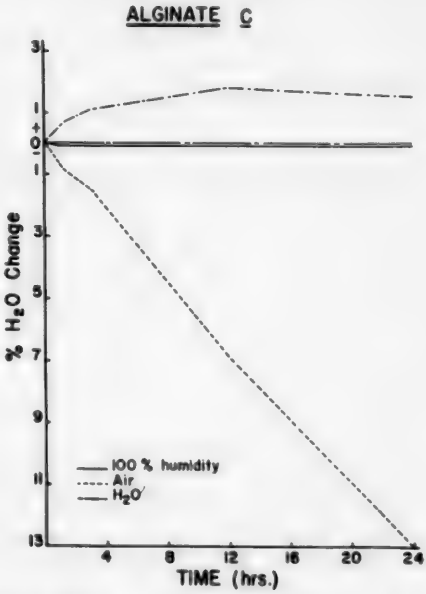

Fig. 3.—Water change in irreversible hydrocolloid C in various media.

Storage Media and Temperatures.—Three storage temperatures were used: (1) 98° F., (2) 70° – 75° F., and (3) 34° F. Two storage media, distilled water and approximately 100 per cent relative humidity, were employed at all 3 temperatures. The results for the 2 reversible hydrocolloids stored at the 3 temperatures in distilled water can be seen in Figs. 4 and 5, while the water change in specimens stored in 100 per cent humidity is plotted in Figs. 6 and 7.

Naturally the water change, at any temperature, is much greater in water than in 100 per cent humidity. When the hydrocolloid is stored in 100 per cent humidity, syneresis is greater at mouth temperature. Little difference can be detected between the two lower storage temperatures. However, when water is used as the storage medium, the imbibition was slightly greater at 98° F. with hydrocolloid B, but no significant difference was evidenced at any temperature with hydrocolloid A. It is obvious that the storage temperature does play some part in water fluctuations but that there is no apparent advantage to be gained by use of low temperatures. However, the higher the storage temperature in 100 per cent humidity, the greater is the water change.

Effect of Residual Stress.—The 2 methods of willfully inducing residual stress—inadequate and rapid gelation and use of undercut dies—were used to

take impressions. These actual impressions were then measured for water content when stored in the various media up to 24 hours. Typical results in 1 medium can be seen in Table II. All specimens were stored at 75° ± 2° F. It is obvious that the per cent of water change in the impression, during storage, is essentially the same regardless of whether the material has or has not been

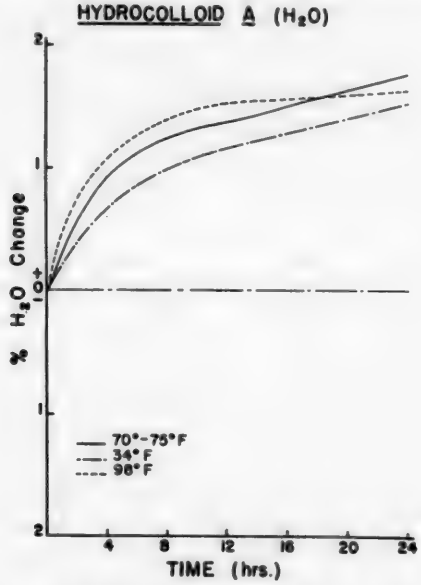

Fig. 4.—Effect of storage of reversible hydrocolloid A in water at various temperatures.

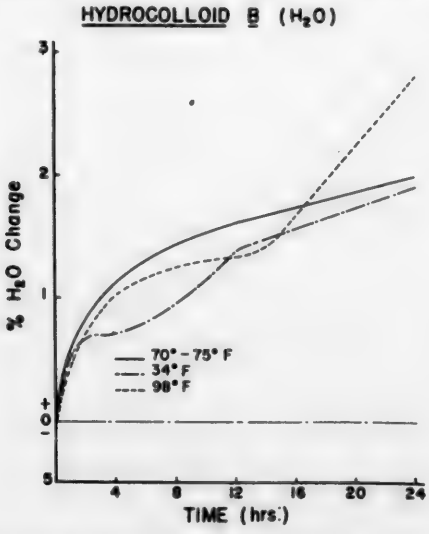

Fig. 5.—Effect of storage of reversible hydrocolloid B in water at various temperatures.

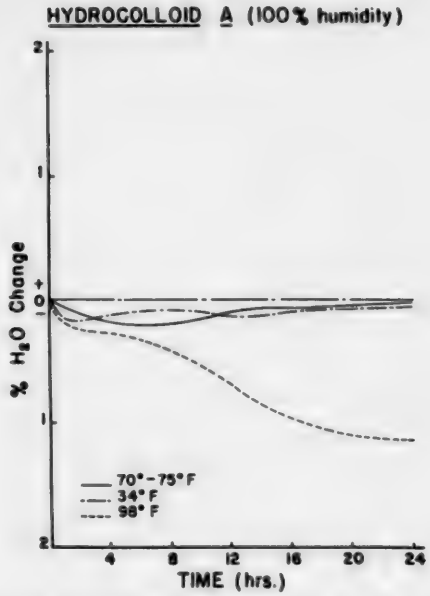

Fig. 6.—Effect of storage of reversible hydrocolloid A in 100 per cent humidity at various temperatures.

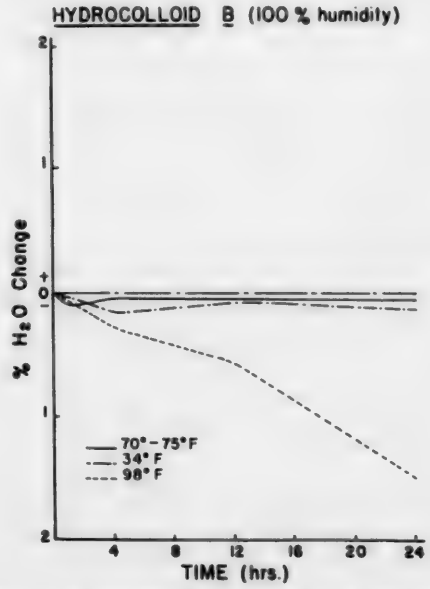

Fig. 7.—Effect of storage of reversible hydrocolloid B in 100 per cent humidity at various temperatures.

handled in a manner designed to increase the residual stress. These data are important since it might be argued that the greater the residual stress, the greater would be the amount of water that would be gained or lost in a given media and, thus, imbibition or syneresis in reality would still be the lone governing factor controlling dimensional change.

The distortion occurring in the control impressions, as compared to that in those which had been improperly gelled or taken from the undercut die, can be

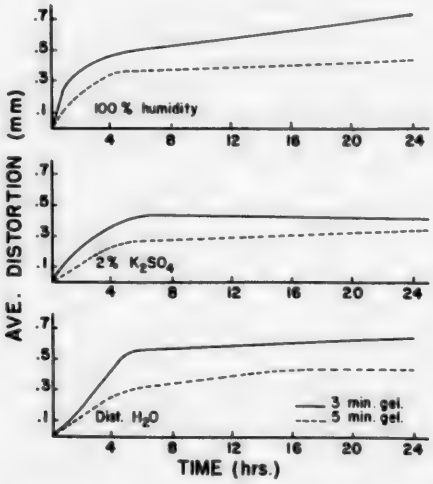

Fig. 8.—Distortion in various media as influenced by stress pattern induced by improper gelation.

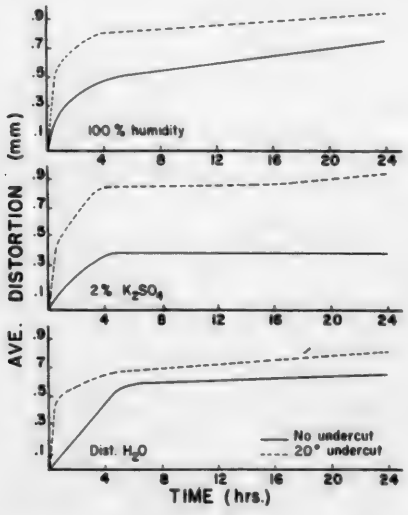

Fig. 9.—Distortion in various media as influenced by stress pattern induced from undercut die.

TABLE II

CHANGE IN WATER CONTENT OF REVERSIBLE HYDROCOLLOID IMPRESSIONS PREPARED IN THE VARIOUS WAYS AND STORED IN 100 PER CENT HUMIDITY AT 75° F.

	½ HR.	4 HR.	24 HR.
5-minute cure	−0.01	+0.15	+0.03
3-minute cure	−0.05	−0.08	+0.08
20° undercut	+0.02	+0.04	+0.10

seen in Figs. 8 and 9. The results with the other hydrocolloid were comparable. The dies were poured immediately after removing the impression at each of the time intervals. The distortion, in 3 different media, is plotted against time. Since the dies poured in impressions taken from the undercut die were not exact reproductions, this original distortion has been subtracted from the subsequent measurement and the corrected values are plotted here. With all specimens, the distortion was greater in impressions which had been subjected to stress than in the controls. The standard deviations on individual specimens were as follows: 5-minute gelation—0.025 mm., 3-minute gelation—0.033 mm., 20° undercut—0.032 mm. It is apparent then that the differences here are greater than the standard deviations in individual specimens.

SUMMARY

The water change in irreversible hydrocolloid impressions when stored in 3 different media was determined. The fluctuation in water content is slightly less in certain media than with reversible hydrocolloid. No significant difference was observed in water change of reversible hydrocolloid impressions when stored at various temperatures, except in 100 per cent humidity where storage at 98° F. increased imbibition.

Technics designed to increase the residual stress in reversible hydrocolloid impressions resulted in greater distortion, even though the water change was the same as in the control. These data suggest that release of internal stress may be of equal importance to water fluctuation during storage.

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STUDIES ON SALIVARIADENECTOMIZED HUNT-HOPPERT CARIES-RESISTANT AND CARIES-SUSCEPTIBLE RATS

I. Effect of Salivariadenectomy on Dental Caries and Several Groups of the Oral Microflora

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BY THE processes of inbreeding, phenotypic selection, and progeny testing, Hunt and Hoppert have developed strains of rats highly susceptible and highly resistant to caries.[1-6] Although these strains have been known for almost 20 years, the hereditary physiological processes responsible for the difference between the 2 lines have not been identified.

A preliminary investigation of the properties of the saliva of these 2 strains revealed that the susceptible animals had a slightly lower viscosity[7] and a much higher proteolytic activity than that of resistant animals.[8] The latter difference was also present in extracts of the submaxillary glands.[9] Before extensive comparisons of the salivas from the 2 strains were undertaken, we wanted to be certain that the absence of saliva would affect the incidence of caries in these rats. While other investigators, using strains of rats not as highly inbred as ours, have shown that salivariadenectomy makes an animal more susceptible to caries,[10-21] Keller, Hunt, and Hoppert found that extirpation of the parotid duct in caries-resistant rats did not significantly decrease their "caries-time."[22] The present study was designed to determine the effect of eliminating the secretion of the major salivary glands on the incidence of caries in resistant and susceptible animals.

EXPERIMENTAL

Both susceptible and resistant rats were kept on a noncariogenic diet* until 60 days old. The submaxillary and major sublingual glands were extirpated and a portion of the parotid ducts removed and the severed ends ligated. Immediately after salivariadenectomy, the rats were placed in individual wire-bottom cages and given the HWC cariogenic ration.†[23-24] Both pair-fed and ad libitum-fed littermates were used. The pair-fed animal was given the same amount of

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*Composition of this diet is as follows: whole powdered milk, 74 per cent; casein, 10 per cent; yeast extract, 5 per cent; alfalfa, 10 per cent; and sodium chloride, 1 per cent.

†This diet now comprises: hulled rice ground so that 1 to 2 per cent is retained on a 20-mesh screen, 66 per cent; whole powdered milk, 30 per cent; alfalfa, 3 per cent; and sodium chloride, 1 per cent.

food daily as its salivariadenectomized littermate consumed on the previous day. When the pair-fed control did not consume as much food as the salivariadenectomized rat, part of the subsequent food allowance was withheld from the pair-fed animal. Food was never withheld from the salivariadenectomized rat.

The method of scoring for caries has been reported previously.[5] A brief description follows. The observations for the identification of carious cavities were made by C. A. Hoppert, and were begun when the rats were about 50 days old. Thereafter, observations were made every 2 weeks. The rat was held ventral side up under a lamp, the jaw was opened with a nasal speculum, and the mandibular molar teeth exposed by pressing back the tongue and cheek. Cavities in these teeth could then be seen. If the existence of the cavity was certain, the date of the observation was taken as the time of appearance of the lesion. If there were one or more successive observations which were doubtful, the last doubtful date immediately preceding the first positive determination was used as the day of first appearance of dental caries. The "caries-time" was the number of days from the date the rat was placed on the cariogenic diet to the day dental caries first appeared.

Microbiologic studies on the oral flora were carried out to determine whether certain groups of microorganisms could be correlated with the production of caries. The method of sampling oral microflora is as follows. Cotton-tipped applicators, 6 inches long, were suspended over 1 ml. of 0.05 per cent aqueous yeast extract in a test tube, and sterilized in an autoclave. The swab was dipped into the yeast extract water just prior to use. Only the right mandibular molars were swabbed because caries generally develop more rapidly in this quadrant of the jaw.[25] The cotton tip of the applicator was carefully broken into a screw-cap tube containing 9 ml. of 0.05 per cent aqueous yeast extract. Any yeast extract water left in the test tube containing the swab was poured into the screw-cap tube. The screw-cap tube was shaken vigorously until the swab disintegrated. Subsequent dilutions were made in distilled water prior to plating. All plates were incubated aerobically at 37° C. The lauryl tryptose broth tubes were incubated for 2 days, tryptone glucose extract agar and crystal-violet azide blood agar plates were incubated for 3 days, and Rogosa SL agar and tomato juice agar plates were incubated for 5 days. All the animals listed in Table I were studied microbiologically.

TABLE I

EFFECT OF SALIVARIADENECTOMY ON CARIES-TIME IN MANDIBULAR MOLARS

GROUP	NUMBER OF RATS	MEAN CARIES TIME AND STANDARD ERROR
Control (Susceptible)	32	54.8 ± 2.6
Salivariadenectomized (Susceptible)	20	47.7 ± 3.0
Control (Resistant)	51	213.1 ± 11.1
Salivariadenectomized (Resistant)	35	104.5 ± 5.9

RESULTS AND DISCUSSION

The results in Table I indicate that salivariadenectomy of the susceptible animals did not have a significant effect on caries. All of the control and salivariadenectomized susceptible animals developed caries. There is a difference of only 7.1 ± 4.0 days in caries-time between control and salivariadenectomized rats. This difference is not significant, as judged by the "t" test given in Table II. The data for the pair-fed and ad libitum control groups were combined, as there was no mean difference between these groups. This accounts for the larger number of control animals than salivariadenectomized animals.

TABLE II

SIGNIFICANCE OF RESULTS ON CARIES-TIME FOR MANDIBULAR MOLARS OF SALIVARIADENECTOMIZED RATS

COMPARISON	DIFFERENCE (DAYS)	*t* VALUE	LEVEL OF SIGNIFICANCE
Salivariadenectomized resistant with control resistant	108.6 ± 12.6	8.6	.000001
Salivariadenectomized susceptible with control susceptible	7.1 ± 4.0	1.8	None
Salivariadenectomized susceptible with salivariadenectomized resistant	56.8 ± 6.6	8.6	.000001
Salivariadenectomized resistant with control susceptible	49.7 ± 6.5	7.7	.000001

Salivariadenectomy had a marked effect on the production of caries in resistant animals. The average caries-time for the salivariadenectomized resistant animals was 104.5 ± 5.9 days, whereas for the controls it was 213.1 ± 11.1 days, a difference of 108.6 ± 12.6 days. Furthermore, the data in Table III show that 91 per cent of the salivariadenectomized resistant animals developed caries in the maxillary molars whereas none of the control resistant animals developed caries in these teeth. Caries in the maxillary molars developed later than in the mandibular molars. Normally, we look for caries in the maxillary molars in older animals only. Therefore, no observations were made for caries in the maxillary molars of susceptible rats because we did not keep them long enough.

TABLE III

EFFECT OF SALIVARIADENECTOMY ON CARIES IN MAXILLARY MOLARS OF CARIES-RESISTANT RATS

GROUP	NUMBER OF RATS	NUMBER WITH CARIES	PER CENT WITH CARIES
Control	42	0	0
Salivariadenectomized	34	31	91

The results in Tables I and II also show that the mean caries-time of the salivariadenectomized resistant animals was significantly longer than for either the salivariadenectomized or control susceptible animals. Although salivariadenectomy made the resistant line more susceptible, the difference between the 2 lines was not obliterated. This indicates that there are inherent traits that contribute to resistance to caries that are *not* contained in saliva. These traits

are probably concerned with morphologic and chemical characteristics of the teeth. It has already been shown that mandibular molar teeth from susceptible rats have greater fissure widths than the teeth from those resistant.[26]

The results of the microbiologic studies on the oral flora of the rats are given in Fig. 1.

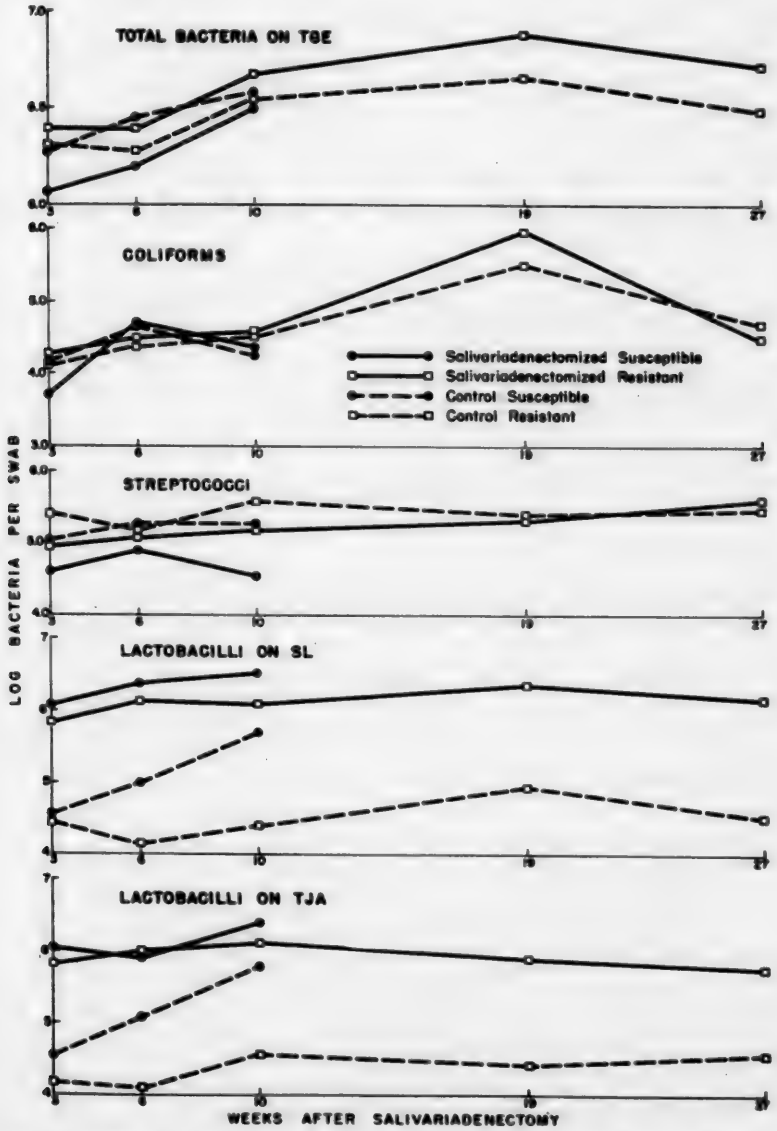

Fig. 1.—Effect of salivariadenectomy on oral microflora. (The animals used at each examination are exactly the same as those given in Table I, except that 52 instead of 51 control resistant rats were used in the examinations at 3 and 6 weeks.)

Tryptone glucose extract agar (TGE—Difco), containing 4 per cent skim milk, was used for an estimation of miscellaneous bacteria in the mouths of these rats. Previous studies in our laboratory showed that this medium gave the highest count of all media used. The results indicate that the total bacteria counted on this medium were about the same for salivariadenectomized and control animals. We attempted to make separate counts for proteolytic bacteria, but they rarely appeared on this medium.

The coliform count varied from one examination period to another, but there was no difference between salivariadenectomized and control animals. The numbers of coliforms were determined by the most probable number technic using lauryl tryptose broth (Difco).

A streptococcal count was made on crystal-violet azide blood agar.* The numbers of streptococci recovered from salivariadenectomized animals were slightly lower than from controls.

Numbers of lactobacilli were determined on 2 media: (1) Rogosa SL agar, modified slightly by substituting half of the glucose with equal amounts of sucrose and arabinose, and (2) tomato juice agar special (TJA—Difco) containing 0.01 per cent sodium azide. Lactobacilli were recovered in higher numbers from salivariadenectomized animals than from controls. At the first examination for oral microflora, there was a high lactobacillus count for the salivariadenectomized animals, which preceded the development of caries. This count remained high for subsequent examinations. The lactobacillus count for control susceptibles showed a gradual increase which paralleled the development of caries.

A statistical comparison of the effect of salivariadenectomy on numbers of lactobacilli on SL agar is given in Tables IV and V. A nonparametric statistical analysis was used because the bacterial populations were not distributed normally and the variances between populations were unequal. The Mann-Whitney "U" test was used because it is a suitable alternative to the parametric "t" test.[27] Since the number of observations in a group was larger than 20, the "z" value was determined from the "U" value.

TABLE IV

STATISTICAL COMPARISON OF EFFECT OF SALIVARIADENECTOMY ON NUMBERS ON LACTOBACILLI

	WEEKS AFTER SALIVARIADENECTOMY	MEAN NUMBER OF LACTOBACILLI PER SWAB × 10^{-3} ON SL AGAR		z
		CONTROL	SALIVARIADENECTOMIZED	
Susceptible	3	37 (32)*	1,138 (20)	7.40
Susceptible	6	91 (32)	2,261 (20)	5.60
Susceptible	10	660 (32)	3,370 (20)	5.24
Resistant	3	28 (52)	671 (35)	7.56
Resistant	6	14 (52)	1,345 (35)	7.78
Resistant	10	26 (51)	1,258 (35)	7.55

*Numbers in parentheses indicate number of animals used.

*This medium comprises: brain heart infusion agar (Difco), 52 Gm., agar, 5 Gm.; crystal-violet, 1 ml. of 0.2 per cent aqueous solution; sodium azide, 100 mg.; and distilled water *q.s.* 1 liter. Defibrinated sheep's blood (4 ml. per 150 ml. media) is added to cool, melted agar prior to use.

TABLE V

STATISTICAL COMPARISON OF EFFECT OF SALIVARIADENECTOMY ON NUMBERS OF LACTOBACILLI

WEEKS AFTER SALIVARIADENECTOMY	MEAN NUMBER OF LACTOBACILLI PER SWAB × 10⁻³ ON SL AGAR		z
	CONTROL SUSCEPTIBLE	SALIVARIADENECTOMIZED RESISTANT	
3	37 (32)*	671 (35)	6.65
6	91 (32)	1,345 (35)	6.53
10	660 (32)	1,258 (35)	3.89

*Numbers in parentheses indicate number of animals used.

The "z" values obtained indicate that the differences in lactobacillus counts between control and salivariadenectomized animals are significant, $p<0.00014$ for each of the 9 comparisons given in Tables IV and V. We conclude that numbers of lactobacilli increase significantly as a result of salivariadenectomy.

An increase in numbers of lactobacilli does not always indicate greater caries activity in this experiment. The results in Table V indicate that the lactobacillus count was higher in the salivariadenectomized resistants than in the control susceptibles. However, caries developed more rapidly in the latter group of animals (Tables I and II). On the other hand, the lactobacillus count was higher in the salivariadenectomized resistants than in control resistants (Table IV) and caries developed more rapidly in the former group of animals (Tables I and II).

In addition to the effects on the incidence of dental caries and the increase in lactobacilli, removal of the salivary glands had marked effects on the dietary habits of these animals.

The body weights of the salivariadenectomized animals were less than the weights of their pair-fed or ad libitum controls. This occurred despite the fact that the operated rats consumed more food per day than their pair-fed littermate controls, and indicated some disturbance in the absorption or utilization of food. The loss of the salivary secretions in the operated rats led to a dryness of the oral mucosa which made mastication difficult. To alleviate this condition, the salivariadenectomized animals drank more water than their pair-fed or ad libitum controls. When swabbing the teeth for microbiologic studies, it was observed that there was greater food retention in the mouths of the salivariadenectomized rats than in their respective nonoperated control animals.

TABLE VI

EFFECT OF SALIVARIADENECTOMY ON FOOD AND WATER CONSUMPTION OF CARIES-SUSCEPTIBLE RATS

	14 WEEKS AFTER SALIVARIADENECTOMY					
	SEX	NO. RATS	BODY WEIGHT (GM.)	WEIGHT GAIN (GM.)	FOOD CONSUMED (GM./DAY)	WATER CONSUMED (ml./DAY)
Ad libitum control	♂	4	300	185	15.1	26
Ad libitum control	♀	8	189	93	11.7	21
Pair-fed control	♂	9	306	182	14.5	25
Pair-fed control	♀	11	178	89	11.0	21
Salivariadenectomized	♂	9	271	145	14.7	34
Salivariadenectomized	♀	11	179	87	11.9	29

Furthermore, for a given 24-hour period, the salivariadenectomized animals took about 3 times as long to consume the same amount of food as the control animals.

The summarized data on food and water consumption, and body weight are given in Tables VI and VII. A detailed report of the changes in these dietary habits will be submitted for publication later.

TABLE VII

EFFECT OF SALIVARIADENECTOMY ON FOOD AND WATER CONSUMPTION OF CARIES-RESISTANT RATS

			14 WEEKS AFTER SALIVARIADENECTOMY				42 WEEKS AFTER SALIVARIADENECTOMY			
GROUP	SEX	NO. RATS	BODY WEIGHT (GM.)	WEIGHT GAIN (GM.)	FOOD CONSUMED (ml./DAY)	WATER CONSUMED (ml./DAY)	BODY WEIGHT (GM.)	WEIGHT GAIN (GM.)	FOOD CONSUMED (GM./DAY)	WATER CONSUMED (ml./DAY)
Ad libitum control	♂	12	374	211	16.7	31	444	281	16.8	33
Ad libitum control	♀	7	225	109	13.0	27	269	153	13.2	34
Pair-fed control	♂	20	332	183	14.5	26	409	260	14.3	27
Pair-fed control	♀	12	211	96	11.3	21	230	115	11.4	27
Salivariadenectomized	♂	22	324	170	16.1	35	362	208	15.1	42
Salivariadenectomized	♀	13	202	85	12.8	31	232	115	12.5	42

SUMMARY

Salivariadenectomy caused an increase in caries in resistant but not in susceptible rats. Salivariadenectomy did not make the resistant line as susceptible to caries as susceptible controls or as salivariadenectomized susceptible rats.

Salivariadenectomy resulted in an increase in lactobacilli but not in other groups of microorganisms.

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CHEMICAL AND PHYSICAL STUDIES OF ENAMEL FROM HUMAN TEETH

I. Specific Gravity and Nitrogen Content of Enamel From Different Surfaces

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IT IS a well-established clinical finding[1, 2, 3] that the mesiodistal enamel surfaces of teeth show a higher incidence of dental caries than the buccolingual surfaces. Although these known differences in distribution of decay between the tooth surfaces are believed to be of external origin, very little attention has been given to the possible variations in the density and nitrogen content of the enamel at the commonly attacked mesiodistal and less susceptible buccolingual surfaces. The absence of consolidated opinion on these subjects prompted this study of sound enamel from teeth of known age and history in order to establish: (1) The possible variations in the density and organic content of sound enamel taken from different surfaces of individual teeth. (2) The distribution of organic material in sound enamel taken from carious and noncarious teeth. (3) The changes in the density and nitrogen content of enamel with age.

MATERIAL AND METHODS

Tooth Specimen.—Only premolar and molar teeth of known age were used in this study. As soon as possible after extraction the teeth were numbered, scraped with dental scalers, and treated lightly with engine brush and pumice to remove all superficial debris, calculus, and plaques. The teeth were classified into six age groups: (1) unerupted teeth, (2) erupted 0-10 years, (3) erupted 11-20 years, (4) erupted 21-30 years, (5) erupted 31-40 years, and (6) erupted over 40 years.

Thereafter, the teeth in each group were grossly examined and the findings for each tooth were recorded on a standardized record sheet. The description included the age of the patient, the number of the tooth, its posteruptive age, enamel surfaces showing clinical changes such as brown spots, white spots, developmentally defective areas, and mottling. The positions of abnormal appearances on tooth surfaces were also noted. The teeth were then stored in distilled water under refrigeration until used.

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Collection of Enamel Samples (Fig. 1).—

The enamel samples used in this study were obtained by cutting several mesiodistal slices from the tooth specimen; the specimen holder in the machine was then turned at right angles so as to cut buccolingual slices. The enamel was separated from each tooth slice by means of the Airdent machine. This was done by directing a fine jet of abrasive on the enamel side of the dentino-enamel junction all around the tooth slab, thus avoiding the risk of contamination with dentin particles. The mesiodistal and buccolingual enamel pieces

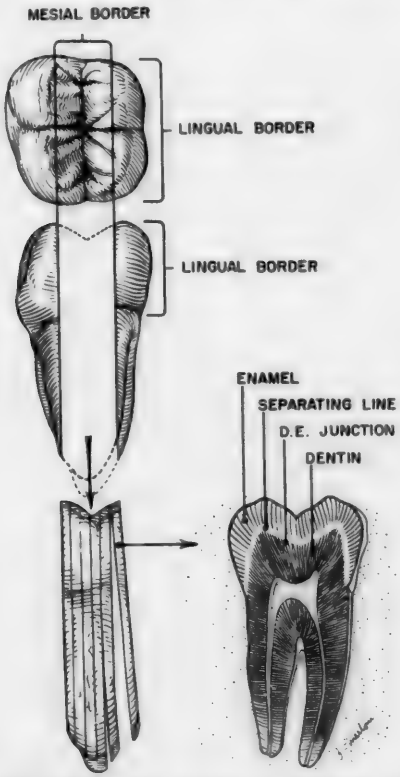

Fig. 1.—Illustration showing the technic for collection of enamel samples.

thus separated were thoroughly washed in distilled water to remove all abrasive particles. They were then dried to constant weight in the oven at 110° C. and used for density and nitrogen determinations.

Physical and Chemical Determinations.—

The micropycnometric technic of Melon and Dallemagne[4] was used for density determinations. The pycnometer had a capacity of 1 c.c. and weighed 2 to 4 grams.

The nitrogen content of enamel was determined by the standard semi-micro-Kjeldahl procedure.[5]

RESULTS

Comparison of the density of enamel from buccolingual and mesiodistal surfaces is shown in Table I. The observations on buccolingual enamel suggest a trend of a slightly higher density than that of mesiodistal enamel at similar ages. This trend is, however, not seen in all groups. For example, there are no density differences between enamel samples from these surfaces when comparing unerupted teeth and those in the 21- to 30-year group. The higher density in the over 40-year group on the other hand may be without significance because of the smaller number of samples in this group.

TABLE I
SOUND ENAMEL: DENSITY (GM./CM.^{3})
BUCCOLINGUAL VS. MESIODISTAL SURFACES

AGE OF TEETH	BUCCOLINGUAL			MESIODISTAL		
	NO. OF TEETH	MEAN	S.E.*	NO. OF TEETH	MEAN	S.E.*
Unerupted	15	2.82 ± 0.07		14	2.86 ± 0.08	
0-10 yr.	25	2.91 ± 0.06		22	2.76 ± 0.06	
11-20 yr.	48	2.82 ± 0.04		47	2.72 ± 0.04	
21-30 yr.	34	2.89 ± 0.05		33	2.86 ± 0.05	
31-40 yr.	15	2.85 ± 0.07		10	2.74 ± 0.09	
Over 40 yr.	18	2.68 ± 0.07		5	2.92 ± 0.13	

*The standard errors are based on the within group standard deviations (pooled estimates), which are as follows: density, 0.29; nitrogen content per gram, 0.32.

The nitrogen content of these enamel samples is presented in Table II. It is apparent that the enamel at buccolingual surfaces has a lower nitrogen content than that at the mesiodistal surfaces except in the 31- to 40-year group. It can, therefore, be concluded from these data that there are indications of a lower density and higher nitrogen content in mesiodistal enamel than in buccolingual enamel at similar ages.

TABLE II
SOUND ENAMEL: NITROGEN CONTENT ON THE BASIS OF WEIGHT (MG./GM.)
BUCCOLINGUAL VS. MESIODISTAL SURFACES

AGE OF TEETH	BUCCOLINGUAL			MESIODISTAL		
	NO. OF TEETH	MEAN	S.E.*	NO. OF TEETH	MEAN	S.E.*
Unerupted	15	0.37 ± 0.08		13	0.48 ± 0.09	
0-10 yr.	25	0.41 ± 0.06		22	0.53 ± 0.07	
11-20 yr.	53	0.39 ± 0.04		47	0.69 ± 0.05	
21-30 yr.	36	0.44 ± 0.05		33	0.53 ± 0.06	
31-40 yr.	23	0.66 ± 0.06		10	0.69 ± 0.10	
Over 40 yr.	25	0.58 ± 0.06		4	0.96 ± 0.16	

*The standard errors are based on the within group standard deviations (pooled estimates), which are as follows: density, 0.29; nitrogen content per gram, 0.32.

The density and nitrogen content of sound enamel from carious and noncarious teeth are summarized in Table III. It is evident that, although there are no significant differences in the density of enamel in these groups, the enamel from sound teeth has a higher nitrogen content than the sound enamel from carious teeth. It is interesting to note that a comparison of values from individual surfaces seems to suggest that the sound enamel from the mesiodistal surfaces of carious teeth has a higher density and a lower nitrogen content than that from the noncarious teeth. It should be pointed out that

in view of the smaller number of samples used in the latter group, these studies are being extended.

TABLE III

SOUND ENAMEL FROM SURFACES OF SOUND AND CARIOUS TEETH

SURFACES	DENSITY (GM./CM.^{3}) NO. OF TEETH	MEAN	S.E.*	N_2 CONTENT (MG./GM.) NO. OF TEETH	MEAN	S.E.*	N_2 CONTENT (MG./CM.^{3}) NO. OF TEETH	MEAN	S.E.*
Sound Teeth									
Buccolingual	35	2.87 ± 0.05		32	0.46 ± 0.06		32	1.39 ± 0.17	
Mesiodistal	24	2.80 ± 0.06		24	0.57 ± 0.06		24	1.63 ± 0.19	
Buccolingual and mesiodistal combined	59	2.84 ± 0.04		56	0.58 ± 0.04		56	1.50 ± 0.13	
Carious Teeth									
Buccolingual	29	2.85 ± 0.05		27	0.38 ± 0.06		27	1.19 ± 0.18	
Mesiodistal	7	3.01 ± 0.11		7	0.43 ± 0.12		7	1.33 ± 0.36	
Buccolingual and mesiodistal combined	36	2.88 ± 0.05		34	0.39 ± 0.05		34	1.22 ± 0.16	

*The standard errors are based on the within group standard deviations (pooled estimates), which are as follows: density, 0.29; nitrogen content per gram, 0.32.

DISCUSSION

The higher nitrogen content found in enamel from mesiodistal surfaces as compared with that from buccolingual surfaces is somewhat difficult to explain. That such differences were found in unerupted teeth introduces the possibility that this is a developmental phenomenon related to tooth formation. However, since the number of unerupted teeth examined was small, it seems worthwhile to consider other factors which might contribute to such difference in the older teeth. The possibility of an analytical artefact due to difference in the size of the samples can be eliminated, because there were no appreciable or systematic differences in this respect between the samples from the proximal or buccolingual regions of the tooth. The possibility that plaque accumulations on the surface could contribute significantly to these variations is not tenable because the differences were noticeable pre-eruptively and because, on all erupted teeth, the superficial debris and plaques were removed by scaling and light brushing with pumice. It is conceivable that lamellae or cracks, which might become filled with extraneous organic material, may be more numerous in the interproximal than in the buccolingual surfaces. This would appear unlikely in view of Gottlieb's observations[8] that such structures are uniformly distributed on all surfaces. Another possibility is that the enamel cuticle may persist for a longer time in the protected interproximal areas than on the exposed buccolingual surfaces, and that the presence of the cuticle on interproximal enamel might account for the increased nitrogen content of this area. In view of the findings of Losee and Hess,[9] that the protein content of enamel proper is 0.22 per cent by weight while that of the cuticle is 0.08 per cent, it would seem that the presence or absence of the cuticle could scarcely account for differences of the magnitude shown in the present data. It should be mentioned that although there is an increase in the nitrogen content of both buccolingual and mesiodistal surface enamel with increasing age, this does not

appreciably alter the differences already present in the nitrogen content of these surfaces pre-eruptively. It can therefore be concluded that the differences between the two areas are fundamentally related to developmental phenomenon.

The finding of a higher nitrogen content in the enamel from the proximal surfaces further raises the question whether this is the reason that these areas are more susceptible to dental caries than are the buccolingual surfaces. That the higher organic content may not be the determining factor in their caries susceptibility is indicated by the findings (Table III) on the noncarious teeth where the organic content of the enamel in the mesiodistal surfaces is somewhat higher than in carious teeth. Thus, it would appear that in so far as the incidence of caries may be influenced by the organic content, the higher organic content cannot be strictly correlated with greater susceptibility to caries. In any event, it must be recognized that local environmental factors may play a significant role in determining the incidence of caries.

Regarding the changes in enamel with age, it is obvious from the present data that the organic content of enamel increases with age in both the buccolingual and mesiodistal surfaces. This supports our previous conclusions[6, 7] of the age changes in enamel.

SUMMARY

1. Density and nitrogen determinations on enamel samples obtained from tooth slices by using an Airdent machine were carried out by Melon's micropycnometric technic and a semimicro-Kjeldahl procedure, respectively.

2. Density and nitrogen determinations on 286 samples from sound teeth suggested that enamel from the buccolingual surfaces (155 samples) had a higher density and lower nitrogen content than enamel from the mesiodistal surfaces (131 samples) of teeth from similar age groups.

3. Sound enamel from noncarious teeth had a higher nitrogen content than sound enamel from carious teeth; these differences were reflected in the mesiodistal surfaces of the noncarious teeth. Marked density differences were not observed.

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TARNISH OF CERTAIN DENTAL ALLOYS

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ONE of the problems associated with dental materials is the tarnish and corrosion of metallic restorations. Corrosion has been defined as a gradual chemical or electrochemical attack of a metal by the atmosphere, moisture, or other agents, while tarnish is considered to be a surface discoloration due to the formation of a thin film of oxide or sulfide.[1] Even though this technical difference does exist, clinically it is difficult to differentiate between the two phenomena and the terms are usually used interchangeably in the dental literature.

Resistance to tarnish and corrosion in mouth fluids has always been considered an important requirement of a good restorative material. Early workers attributed discoloration of dental restorations and the surrounding tooth tissue to the formation of various metallic sulfides. It has been noted that dental alloys tarnished more in some mouths where good oral hygiene appeared to be practiced than in the mouths of patients whose hygiene was less meticulous.[2, 3] Likewise, it has been suggested that ingested foodstuffs, medicines containing iron, mercury, and sulfur, and differences in the alkalinity of saliva might all play an important role.[3, 4]

Corrosion has been attributed to factors such as the composition and structure of the alloy and differences in electrical potential due to a lack of homogeneity of the alloy or to external contact with other metals.[5, 6, 7] Schoonover and Souder[6] noted a loss in luster of amalgam specimens placed in a 1 per cent NaCl solution. The loss of luster by the amalgam was accelerated when specimens of gold alloys where placed in close proximity or in direct contact with amalgam. A sulfurized oil bath has been used as a method of determining the resistance of gold alloys to tarnish. Alloys with a precious metal content below 75 per cent tarnish by this test and hence are not considered sufficiently tarnish resistant for restorative purposes.[7] Unfortunately, there is no clinical corroboration of this laboratory test and there is no accepted method for determining the relative susceptibility of amalgam alloys to tarnish.

It was the purpose of this investigation to determine the chemical nature of tarnish produced in vivo and to study the effect of certain variables upon in vitro tarnish.

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PROCEDURE

In Vivo Tarnish.—In order to determine something of the chemical nature of the tarnish occurring on clinical restorations, x-ray diffraction patterns were obtained on heavily tarnished amalgam restorations removed from freshly extracted teeth. These patterns were then compared with a second series of patterns made on the restorations after the tarnish had been removed.

In Vitro Tarnish.—Two representative commercial amalgam alloys, one containing zinc and the other a nonzinc alloy, an experimental gallium alloy* and two gold alloys,† one high and one low karat, were used in the study. Tarnish was studied by suspending discs of each alloy in the various media.

Unless otherwise stated, all amalgam specimens were prepared at the recommended 8:5 mercury-alloy ratio and triturated by a mechanical amalgamator. The specimens were condensed into plastic molds under a static load of 4,000 lbs. per square inch applied for 2 minutes. All amalgam specimens were trimmed flush with the top surface of the mold immediately following condensation. When the effects of polishing were studied, the specimens were polished by conventional dental procedures, 24 hours after condensation.

The effect of residual mercury was studied by a series of specimens prepared with 3 different mercury-alloy ratios—5:5, 5:8, and 5:12. As a control, an ingot† of a typical dental alloy (65 per cent silver, 28 per cent tin, 5 per cent copper, and 2 per cent zinc) was also used in the study.

The gallium specimens were prepared from a liquid, 89 per cent gallium and 11 per cent tin, and a powder containing 62 per cent copper and 38 per cent tin. They were condensed in the plastic molds and trimmed in the same manner as the amalgam specimens.

The tarnish of gold alloys with a high noble metal content was studied by suspending pennyweights of a typical commercial dental alloy in various media. The alloy was Type B containing a minimum of 78 per cent of the gold-platinum group metals. The low karat gold alloy used in the experiment was a gold solder, 0.490 fine. Strips of the solder were suspended in the media.

The ions selected for use in this study were those present in saliva or in foodstuffs which were most apt to react with the metals to form colored compounds. The concentration ranges employed were selected in order to simulate the quantities present in saliva as well as amounts found in some of the common foods. The media can be seen in Table I. Thirteen different media and a total of 958 specimens were employed in the investigation.

The tarnish was studied by suspending specimens for 66 days in 30 ml. of the solutions. The solutions were changed weekly. Any loss of solution due to evaporation was replaced by restoring the original volume with distilled water. With hydrogen peroxide, the solution was replenished by the addition of a quantity of fresh solution at the appropriate concentration.

*Furnished by the Dental Section, National Bureau of Standards, Washington, D. C.
†Furnished by The J. M. Ney Company, Hartford, Conn.

TABLE I
TEST MEDIA USED

SULFIDE (% SODIUM SULFIDE)	CHLORIDE (% SODIUM CHLORIDE)	OXIDE (% HYDROGEN PEROXIDE)	MISCELLANEOUS
1. 0.005			
2. 0.05	*5.* 0.05	*8.* 0.05	*11.* Air
3. 0.5	*6.* 0.5	*9.* 0.5	*12.* Distilled water
4. 5.0	*7.* 5.0	*10.* 6.0	*13.* Synthetic saliva

The degree of tarnish was assessed by visual examination of the surfaces. Tarnish was divided into 8 categories and a numerical value assigned to each. Where no change in the surface was observed a value of 0 was assigned. A very slight discoloration of the surface was scored as 1 while a deep gray was assigned a value of 4. Completely black surfaces were scored as 7. Appropriate numerical values were assigned to intermediate degrees of tarnish. Ratings of 1 and 2 were considered light or slight tarnish. Gradings of 3 and 4 were evaluated as medium, while 5, 6, and 7 were heavy. The specimens were examined daily and the progress of tarnish charted by means of these numerical

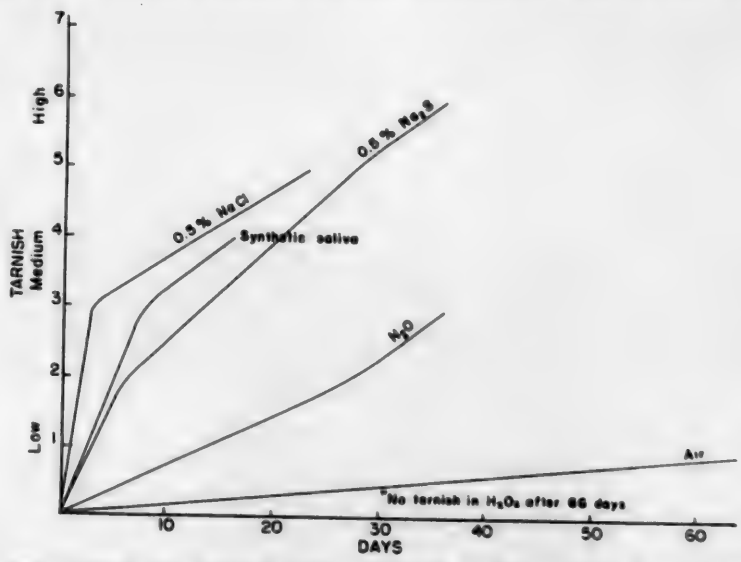

Fig. 1.—Rate and maximum degree of tarnish of zinc amalgam in various media.

values. It is realized that this method of evaluation might be considered somewhat more qualitative than quantitative. All evaluations, however, were made by the same investigator and repeated scoring of large groups of specimens proved his ability to determine the degree of tarnish with consistency. The acceptability of this method was further verified by comparison of series of photographs made of test specimens stored in the various solutions over periods of time. These visual observations were augmented by microscopic examinations.

RESULTS

In Vivo Tarnish.—The results of these studies indicate the amalgam alloy system to be quite complex with some unidentifiable x-ray diffraction lines. On one specimen, a complex sulfide with a tentative formula of $(Hg,Ag)S_x$ was observed while a silver sulfide and a mercury sulfide, in the form of cinnibar, were identified on another specimen. Tarnish appeared to be principally

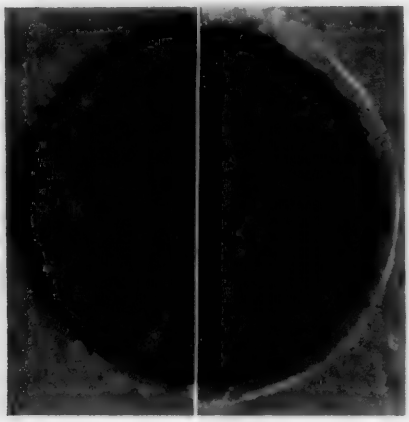

Fig. 2.

Fig. 3.

Fig. 2.—Amalgam specimens stored in 0.5 per cent Na_2S solution. *Left*, 16 days (tarnish rating 4). *Right*, 32 days (tarnish rating 7). (Mag. ×7; reduced ¼.)
Fig. 3.—Amalgam specimen stored 32 days in 0.05 per cent Na_2S (tarnish rating 3). (Mag. ×7; reduced ¼.)

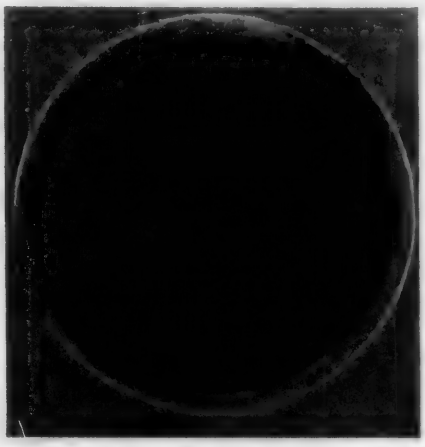

Fig. 4.—Amalgam specimen stored 32 days in 0.5 per cent NaCl (tarnish rating 5). (Mag. ×7; reduced ¼.)

Ag_2S on a third specimen. Although there were these differences in the exact compositions of the sulfide compounds, the results all indicate that in vivo tarnish of amalgam is primarily due to sulfur.

Additional information that amalgam tarnish is partially a mercuric sulfide was obtained through microscopic examination of the specimens. Although generally the tarnished amalgam is black to the naked eye, when viewed under magnification there are red deposits in the pits and depressions. Alpha cinnibar is red in color.

In Vitro Tarnish.—In order to conserve space, all the voluminous data collected in this investigation could not be included in this paper. Only the more pertinent observations will be presented.

All specimens were stored and examined over the 66-day test period. However, the progress of tarnish has been plotted on the graphs only up to the time at which the highest level of tarnish was reached for each type specimen in a particular medium. The effects of NaCl, Na_2S, and H_2O_2 are represented only by the data obtained with 0.5 per cent solutions although the results with other concentrations are discussed.

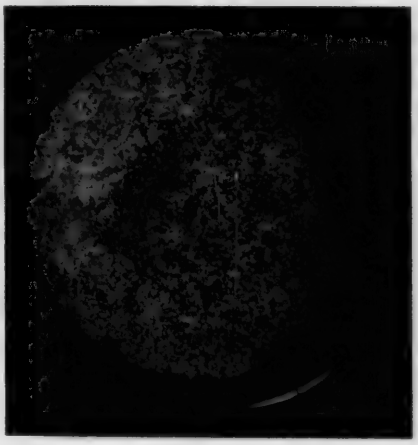

Fig. 5.

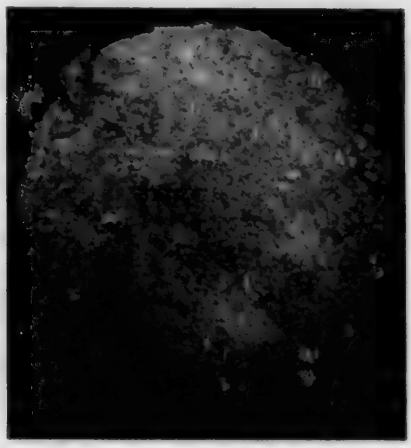

Fig. 6.

Fig. 5.—Amalgam specimen stored 66 days in air (tarnish rating 1). (Mag. ×7; reduced ¼.)

Fig. 6.—Amalgam specimen stored 66 days in 0.5 per cent H_2O_2 (tarnish rating 0). (Mag. ×7; reduced ¼.)

Media.—The maximum degree and rate of tarnish of amalgam specimens stored in various media are shown in Fig. 1. The curves are the mean values obtained with 8 specimens.

High degrees of tarnish were produced by the sulfide solutions. An average of 36 days was required for the specimens to reach maximum tarnish in 0.5 per cent Na_2S with no further change detected. Concentration appeared to influence the severity of tarnish as specimens immersed in the 0.005 per cent and 0.05 per cent solutions exhibited less discoloration than those exposed to either the 0.5 per cent or 5.0 per cent solutions. Some specimens stored at the higher concentrations evidenced such gross discoloration that they were assigned the

highest tarnish rating of 7 (Fig. 2). A specimen immersed for 32 days in 0.05 per cent Na_2S can be seen in Fig. 3. The appearance of specimens subjected to a 0.005 per cent concentration was comparable.

A high degree of tarnish was also exhibited by specimens immersed in solutions of NaCl. A maximum tarnish of 5 was noted with specimens immersed in each of the 3 concentrations. This level was reached in 23 days in the 5.0 per cent and 0.5 per cent solutions but not until 29 days in the 0.05 per cent NaCl. The surface of a specimen after 32 days in 0.5 per cent NaCl is shown in Fig. 4.

Medium tarnish was observed on the surfaces of specimens stored in water and synthetic saliva. Discoloration progressed slower and was less in water than in saliva. After 16 days in synthetic saliva, specimens reached a maximum tarnish of 4, while in water the increase was gradual over a 36-day period until a figure of 3 was assigned. When the experiment was terminated at 66 days there had been no further change in the surface discoloration.

After 66 days only a very slight tarnish appeared on surfaces exposed to air (Fig. 5) while no change was detected in the H_2O_2 solution (Fig. 6).

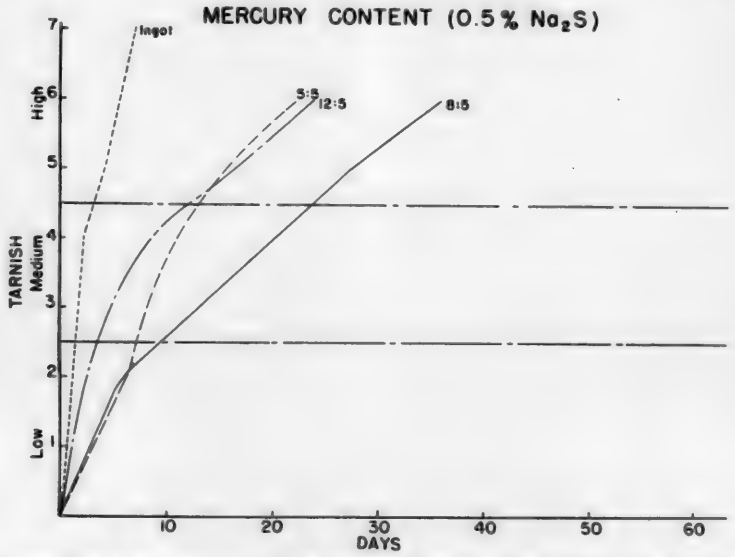

Fig. 7.—Rate and maximum degree of tarnish of zinc amalgam specimens containing various quantities of mercury. Ingot contained no mercury and was included for comparison.

Effect of Mercury.—The tarnish of amalgam specimens with varying percentages of mercury stored in a 0.5 per cent Na_2S solution can be seen in Fig. 7. No correlation between tarnish and the amount of mercury in the specimen could be detected in any of the media used. In fact, the ingot, which contained no mercury at all, demonstrated a more rapid and greater tarnish than did specimens which contained mercury. It tarnished more in 9 out of the 13 media included in the study.

Polish.—The tarnish of amalgam specimens, polished and unpolished, are plotted in Fig. 8. Both polished and unpolished specimens exhibited heavy

tarnish in a 0.5 per cent Na_2S solution. Although the ultimate tarnish was as great, polished surfaces did not tarnish as rapidly in Na_2S solutions as did unpolished ones. Unpolished amalgam specimens were assigned tarnish ratings of 6 after 36 days, while 56 days elapsed before a comparable discoloration was noted on the polished surfaces. Examination of data for all media indicated this general trend.

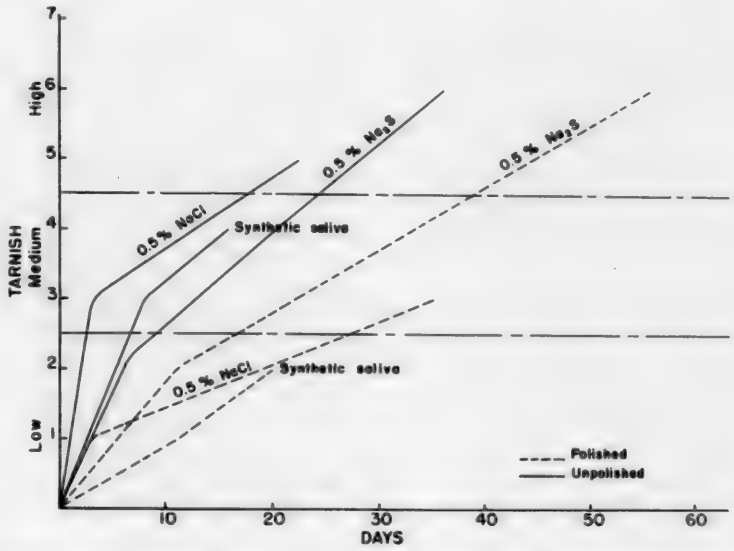

Fig. 8.—Rate and maximum degree of tarnish of polished zinc amalgam specimens compared to unpolished specimens in various media.

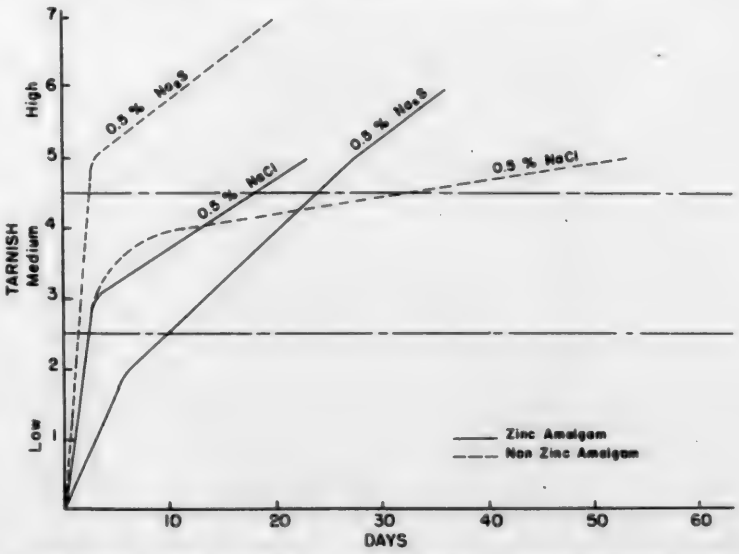

Fig. 9.—Rate and maximum degree of tarnish of nonzinc amalgam compared to zinc amalgam in various media.

Zinc and Nonzinc Amalgam.—The results obtained with a zinc and nonzinc amalgam, from the same manufacturer, are shown graphically in Fig. 9. The nonzinc alloy tarnished to the maximum limit in only 20 days in a 0.5 per cent Na_2S solution, while the zinc alloy reached a maximum tarnish of 6 at the end of 36 days. Nevertheless, the complete data did not indicate a measurable difference in the susceptibility of the two alloys to tarnish. Nonzinc alloys produced high tarnish in 7 media, while zinc amalgam was graded high in 6 solutions. Medium degrees of tarnish of the nonzinc alloy were noted in 2 solutions while the zinc alloy was rated as medium in 3 media. Low ratings, or no tarnish at all, were found with both alloys in 4 different media.

In order to determine whether these results were indicative of the behavior of nonzinc alloys in general or peculiar to this alloy, similar tests were conducted with a second commercial nonzinc alloy. Comparable results were obtained. Hence, these data seemed to indicate that the presence or absence of zinc in a dental alloy will have little influence on its tendency to tarnish.

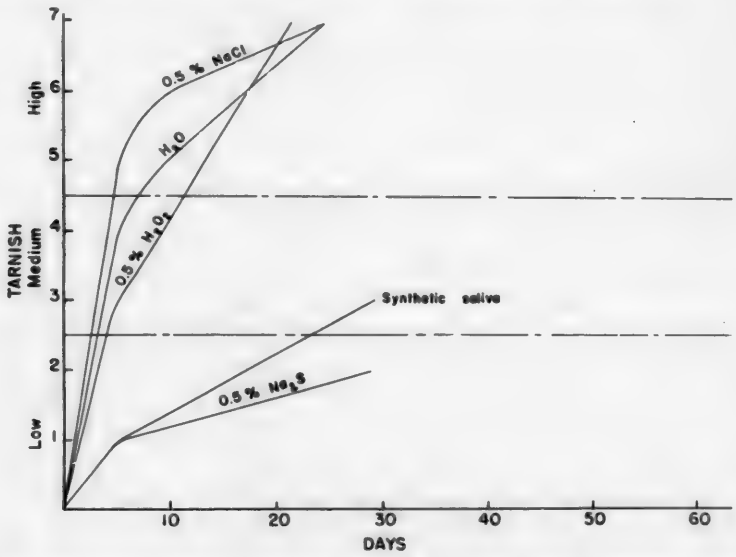

Fig. 10.—Rate and maximum degree of tarnish of gallium alloy specimens stored in various media.

Gallium.—The results with the gallium alloys are presented in Fig. 10. It is interesting to note that exposure to sulfides, which invariably resulted in gross tarnish of amalgam, produced comparatively little discoloration on gallium alloy surfaces. However, hydrogen peroxide, which had no effect on any of the amalgam specimens, produced marked tarnish of the gallium alloy. All specimens immersed in this medium were assigned the maximum tarnish number of 7. Complete tarnish of these specimens was also seen in sodium chloride solutions and in distilled water. Examples of gallium specimens stored in distilled water, synthetic saliva, and 0.5 per cent Na_2S appear in Figs. 11, 12, and 13.

Although sulfides did not affect the gallium alloy—chlorides, oxygen, and even moisture resulted in a rapid and gross discoloration of this alloy. It would appear, particularly in view of the data obtained with distilled water, that clinical tarnish or corrosion might be as great a problem with gallium as it is with amalgam.

Gold Alloys.—No tarnish was detected on the surfaces of the high karat gold alloy in any of the 13 media. The gold solder discolored slightly in Na_2S

Fig. 11.

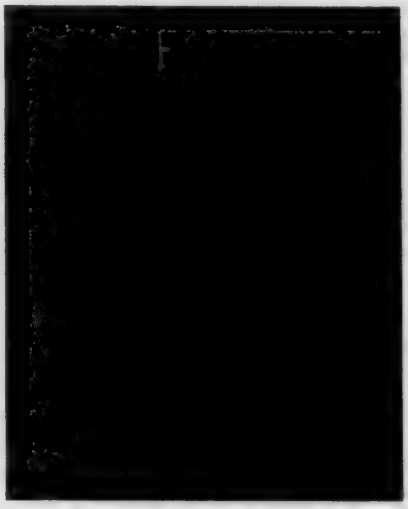

Fig. 12.

Fig. 11.—Gallium alloy specimen stored 32 days in distilled water (tarnish rating 7). (Mag. ×7.)

Fig. 12.—Gallium alloy specimen stored 32 days in synthetic saliva (tarnish rating 3). (Mag. ×7.)

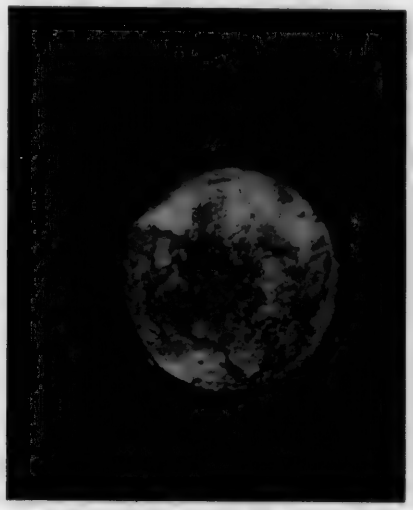

Fig. 13.—Gallium alloy specimen stored 32 days in 0.5 per cent Na_2S solution (tarnish rating 2). (Mag. ×7.)

solutions at the end of 18 days. It was scaled at 2 at that time and no further discoloration was noted. These results are in agreement with other investigators who found gold alloys with a low precious metal content discolored in the presence of sulfides while high karat alloys were unaffected.[7] No change was seen on the surfaces of the solder in any of the other media.

Galvanic Action.—In order to study the manner in which galvanic action might contribute to tarnish and corrosion of materials, gold alloy and amalgam specimens were suspended in the same medium at a distance of 10 mm. There was only slight tarnish of the amalgam specimens while no tarnish could be detected on the surface of the gold alloy specimens. These results are in agreement with observations made by other workers[6] who have noted increased tarnish of amalgam and corrosion of the gold only when the metals were in direct contact or in very close proximity. It is quite possible that if the distance between the materials had been reduced, some effect might have been demonstrated.

Nature of In Vitro Tarnish.—X-ray diffraction patterns were made on some of the specimens after removal from the immersion media. The tarnish film produced on the surface of zinc amalgam stored in a 0.5 per cent Na_2S solution was identified as mercuric sulfide, with smaller amounts of silver and perhaps tin compounds. The diffraction pattern of a grossly discolored gallium alloy removed from a 0.5 per cent NaCl solution suggested cupric oxide as a component of the tarnish. No identification could be made on amalgam surfaces which were discolored by immersion in either artificial saliva or sodium chloride.

DISCUSSION

No attempt was made in this study to differentiate between tarnish and corrosion. However, results indicate the fallacy of attempting to evaluate the susceptibility of alloys to either tarnish or corrosion on the basis of their behavior in a single medium. The tests with the gallium alloy emphasize this point. If the gallium alloy had been observed only in a sulfide medium, the conclusion undoubtedly would have been drawn that it was less subject to tarnish and corrosion than amalgam. However, since gallium alloy specimens discolored in chlorides, hydrogen peroxide, and water as much as amalgam specimens did in sulfide solutions, the implication might be that a gallium alloy would be equally susceptible to tarnish under clinical conditions. The difference would be that the principal constituents of the tarnish produced on gallium alloy restorations would probably be oxides or chlorides rather than sulfides.

It was interesting to note that the mercury content of amalgam is apparently not a governing factor in the tarnish of amalgam alloys. Perhaps this is not too surprising in view of the x-ray diffraction data of both clinically and artificially tarnished specimens which indicated a silver compound to be a major constituent of tarnish. These results are further corroborated by the work of Schoonover and Souder,[6] who measured the electrical potential

of amalgam specimens with different mercury contents and observed differences of such small magnitudes as to rule out a correlation between mercury content and potential. It was their opinion that other factors such as surface condition, strain, etc., produced greater variations in potential than small differences in composition, and hence were more important factors in corrosion. The observations made in this investigation on polished surfaces would seem to support that point of view.

SUMMARY

An investigation was conducted to determine the role of various chemical elements in the tarnish of certain dental alloys.

1. X-ray diffraction patterns of clinically tarnished amalgam restorations indicate sulfide to be a dominating factor in the tarnish of amalgam alloys.

2. Amalgam specimens exhibited gross discoloration in sulfide solutions. Lesser degrees were noted in chlorides, synthetic saliva, and water.

3. The degree and rate of tarnish of the amalgam specimens did not appear to be influenced either by the residual mercury content or by the presence or absence of zinc.

4. Sulfur produced little tarnish on the gallium alloy, although heavy tarnish was incurred by storage in sodium chloride, hydrogen peroxide, and distilled water. It would thus appear that with this alloy, oxides or chlorides would be the principal constituents of tarnish.

5. A light tarnish of a low karat gold alloy was induced by sulfides. This alloy was not affected by other elements. High karat gold was not influenced by storage in any of the media used.

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SALIVARY GLAND FUNCTION IN THE RAT

I. Flow, Viscosity, and pH in the Normal and Duct-Ligated Rat

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REPEATED studies from this laboratory have suggested a significant relationship between experimental dental caries in the rat and the function of various endocrine organs, especially the thyroid gland.[1-6] The mechanism of this relationship is not known, although convincing evidence suggests that the salivary glands are directly involved. The experimental basis for implicating the salivary glands are changes known to occur in both the histologic structure as well as in the enzymatic activity of these glands concomitant with thyroid dysfunction. These findings have been reported previously.[3-6]

Undoubtedly, any alteration in histologic structure of an organ must be reflected in an aberration from normal function of that organ. The estimation of dysfunctions in certain organs is relatively simple and may be adapted to experimental investigations in even small laboratory animals. In other organs, especially where a diversity of function exists, the estimation of changes from the normal may be difficult. Because of the almost certain relationship between endocrine dysfunction and salivary gland activity, and the obvious implications regarding dental caries activity, it became of interest to investigate salivary function.

The present investigation deals with a series of base-line studies designed in order to determine the accuracy, reproducibility, and applicability of salivary function tests (flow, viscosity, pH) to current endocrine studies in this laboratory.

EXPERIMENTAL

In this study, salivary flow was stimulated by the subcutaneous administration of 10 mg. of pilocarpine nitrate per kilogram of body weight to rats previously anesthetized by intraperitoneal injections of sodium Nembutal (35 mg. per kilogram of body weight with the concentration of Nembutal being 5 mg. per milliliter of solution). The animals were placed in an apparatus similar to that described by Benarde, Fabian, Rosen, Hoppert, and Hunt.[7] Saliva was collected for 1 hour as it flowed from the mouth, measured in a 1 ml. tuberculin syringe, and recorded as total milliliters of saliva secreted per hour.

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Salivary viscosity was determined by measuring the time needed for a given volume of saliva to flow between two marks, 28 cm. apart, in a thin Pyrex tube microviscosimeter. The viscosity measurements were made on every saliva sample within several minutes after secretion. The salivary pH was measured by means of a one-drop Beckman electrode on saliva taken immediately after secretion.

The first portion of the study consisted of 25 male albino rats divided into 3 groups according to age: weaning (26 days old), young adults (126 days old) and old adults (1 year old). Salivary flow, viscosity, and pH were determined in these animals in order to measure the effect of age on these functions.

The second portion of the study consisted of 40 male albino rats which were 75 to 85 days of age at the beginning of the experiment and which were divided into 5 groups. In 4 of these groups, either the parotid or submaxillary-sublingual salivary ducts were surgically ligated, unilaterally or bilaterally. As a result, it was possible to determine the contribution of either the parotid or the combined submaxillary-sublingual glands to the total volume of saliva secreted. Analytical determinations were carried out in all of the animals 26 days following duct ligation.

As a subordinate part of this study, 6 male rats, 2 months of age, were totally desalivated in order to determine whether the accessory salivary glands in the rat contribute any significant part to the total volume of saliva secreted. Six unoperated animals of the same age served as controls. The analytical determinations on the operated animals were carried out 2 weeks following the surgical procedure.

All animals in these studies were maintained in raised wire bottom cages and received a stock laboratory diet and distilled water ad libitum.

RESULTS

In these studies, salivary flow was found to increase with the age of the animal from a mean of 1.0 ml. per hour at 26 days of age to 3.8 ml. at 1 year (Table I). The viscosity decreased from 75.2 seconds at 26 days to 48 seconds at the end of 1 year. The pH showed a progressive drop from 8.6 units in the

TABLE I

SALIVARY FLOW, VISCOSITY, AND pH IN RATS AS A FUNCTION OF AGE

GROUP	NO. OF ANIMALS	AVERAGE BODY WEIGHT (GM.)	AVERAGE SALIVARY FLOW (ML./HR.)	AVERAGE VISCOSITY (SEC.)	AVERAGE pH
Weanling (26 days old)	10	76	1.0 ± 0.9*	75.2 ± 3.0*	8.6 ± 0.3*
Young adult (126 days old)	9	241	2.8 ± 1.5	49.9 ± 7.7	8.3 ± 0.3
Old adult (1 year old)	6	462	3.8 ± 0.7	48.0 ± 7.7	8.0 ± 0.2

*Standard deviation.

youngest group to 8.3 units in the young adults and 8.0 units in the oldest animals. The flow and viscosity differences between weanlings and old adult animals were significant at the 0.01 level of confidence, and the pH differences at the 0.001 level of confidence.

Blocking the flow of the individual salivary glands by duct ligation has provided evidence of the contribution of each gland to the total saliva in the rat (Table II). Control animals, 100 to 110 days of age, secreted an average of 3.1 ml. of saliva per hour. When the parotid duct was ligated unilaterally and bilaterally, the flow was reduced to 2.0 and 1.7 ml. per hour, respectively. When the combined submaxillary-sublingual ducts were ligated unilaterally and bilaterally, the flow was 1.8 and 1.0 ml. per hour, respectively.

TABLE II

EFFECT OF SELECTIVE SALIVARY GLAND DUCT LIGATION ON SALIVARY FLOW, VISCOSITY, AND pH IN THE RAT

GROUP	NO. OF ANIMALS	AVERAGE BODY WEIGHT (GM.)	AVERAGE SALIVARY FLOW (ML./HR.)	AVERAGE VISCOSITY (SEC.)	AVERAGE pH
Control	7	299	3.1 ± 0.8*	54.5 ± 3.1*	8.1 ± 0.2*
Unilateral parotid ligate	9	224	2.0 ± 1.0	66.9 ± 9.5	8.0 ± 0.1
Bilateral parotid ligate	9	255	1.7 ± 1.1	65.4 ± 10.0	8.2 ± 0.2
Unilateral submaxillary-sublingual ligate	9	228	1.8 ± 1.0	50.1 ± 8.0	7.8 ± 0.1
Bilateral submaxillary-sublingual ligate	6	180	1.0 ± 1.1	27.0 ± 2.5	8.1 ± 0.4

*Standard deviation.

The only significant ($P = 0.001$) difference in viscosity of the saliva occurred when the combined submaxillary-sublingual ducts were ligated bilaterally. This procedure blocked the flow of the highly viscid saliva from the mucous sublingual gland. No significant changes were found in salivary pH.

That portion of the study designed to determine the contribution of the accessory glands to the total flow by extirpation of all major salivary glands indicated that these accessory glands contribute very little saliva to the total flow. Of the 6 desalivated animals, 4 produced no measurable amount of saliva

TABLE III

EFFECT OF EXTIRPATION OF MAJOR SALIVARY GLANDS ON SALIVARY FLOW IN RATS

GROUP	NO. OF ANIMALS	AVERAGE BODY WEIGHT (GM.)	AVERAGE SALIVARY FLOW (ML./90 MIN.)
Control	6	240	2.6 ± 1.5*
Desalivate	6	205	0.2 + 0.4

*Standard deviation.

while 1 animal produced only 0.2 ml. and the other, 1.2 ml. The flow, particularly in the latter animal, may have been the result of faulty surgical technic.

DISCUSSION

The present studies indicate primarily the feasibility of utilizing these technics for investigating the physiologic functions of the rat salivary glands under various laboratory conditions. The study of human salivary gland functions, even under so-called "normal" conditions, is at best a difficult one and one in which unavoidable variables complicate or even completely mask the results. Furthermore, investigation of human salivary gland functions under most experimental conditions which are necessary for the experimental pedagogy of the study is frequently impossible to achieve. For this reason, technics adaptable for use with small animals must necessarily be utilized provided their limitations are recognized.

These studies indicate that salivary flow increases with advancing age but do not clarify whether this is dependent primarily upon the body weight or size of the animal, the weight or size of the salivary glands, the total circulating blood volume, "filtration" factors associated with aging of cells, or any of a number of other possible factors. Because so little is known concerning mechanism of secretion by salivary glands and secretion selectivity and the factors which affect them, an interesting and fundamental area for further investigation exists in such studies.

Despite the fact that all animals were maintained under identical experimental conditions and treated in as nearly an identical manner as possible when determining salivary flow, there was considerable individual variation even between animals within the same group. The reasons for this variation are not known.

Salivary viscosity, a feature which appears unrelated in these studies to the rate of salivary flow, is indicative of the degree of function of the mucous salivary glands in the rat. Its significance with respect to oral health or maintenance of dental structures is entirely unknown. The same statement may be made for salivary pH although this function has been widely studied in the human being.

SUMMARY AND CONCLUSIONS

The salivary flow, viscosity, and pH have been studied in groups of rats at varying ages. Flow was found to increase with advancing age while both viscosity and pH decreased from weaning to adulthood.

Selective salivary gland duct ligation results in diminution in flow of varying proportions depending on the ducts ligated and whether this was a unilateral or bilateral procedure. A significant reduction in salivary viscosity occurred when the submaxillary-sublingual ducts were ligated bilaterally thus blocking flow of the mucous saliva from the sublingual gland. No differences in the pH of saliva from different glands were noted.

Total extirpation of the major salivary glands revealed that the accessory glands in the rat contribute very little to the total salivary flow.

The need for intensive investigation of salivary gland function in the small laboratory animal under controlled conditions is stressed.

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RELIABILITY OF MEASUREMENTS FROM CEPHALOGRAMS AT THE BURLINGTON ORTHODONTIC RESEARCH CENTRE

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CEPHALOMETRY is now generally accepted as a valuable adjunct to the study of anatomy. In 1954, Bjork[1] wrote, "The x-ray method for obtaining skull measurements has facilitated research into the growth and development of the head, the face, and the bite, and has greatly advanced our understanding of these problems." This paper is an exploration of the reliability of specific cephalometric technics being used by the present investigators at the Burlington Orthodontic Research Centre.

At Burlington, the roentgenograms are produced by a high kilovoltage machine with a distance of 60 inches between the target and the subject. For lateral head plates, the distance between the film and the subject (median sagittal plane of skull) is 6 inches. For the oblique plates, the head is rotated, by means of the Thurow head positioner, until the median sagittal plane forms an angle of 43 degrees to the film. This rotation in no way interferes with the target-subject distance or the angle of the central axis ray to the film. (For the anteroposterior plates not used in the present study the forehead and nose touch the casette.)

Aside from the main objective of this paper, it must be noted that the measurements of the radiographic projections or images of anatomic structures are all biased (i.e., they fail to portray the exact size or proportions of the subject), hence particular care is needed in the interpretation and use of cephalometric data. One can readily see that there is a general magnification inherent in the technic but, what is more serious, the presence of distortion (i.e., disproportionate magnification of different structures) must be always kept in mind. The general magnification of the lateral head plates is approximately 10 per cent, depending upon the position of the particular structure in relation to the median plane; similarly for the obliques, the magnification of structures close to the film is less than 10 per cent and obviously increases as the distance between the structures and film increases. Thus, measurements of projections of the same structure taken from different types of head plates are differently biased and cannot be interchanged, nor can measurements from casts be substituted for those from plates. Nevertheless, measurements taken from different

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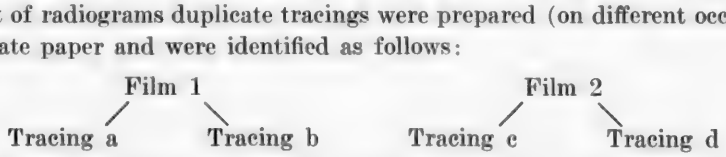
subjects can be used validly in making comparative studies and in constructing norms, if they have been obtained from the same type of head plate, and, if the same or duplicate equipment is used for each patient or group of patients.

Bearing in mind the unavoidable projection distortion, the practical purpose of this study was to examine the presumably random (since it is assumed here that the true average technical errors are zero) technical errors which reduce the precision but do not influence the bias of the cephalometric measurements. Fisher[2] has stated that "refinements of technique . . . contribute nothing to the validity of the experiment, and of the test of significance by which we determine its result. They may, however, be important, and even essential in permitting the phenomenon under test to manifest itself." Cephalometric technical variation arises from minor changes in head orientation in the positioning device, from measuring and tracing procedures (which include the difficulty of identifying structures) and from a residuum of unidentified experimental error. The reliability of an estimate of a mean is equal to the reciprocal of its standard error squared, $\frac{N}{\sigma^2}$, and for cephalometric estimates may be expressed as the real technical variance components $\frac{N}{V + V(r) + V(t)}$ where N is the sample size, V the real variation between subjects plus the unidentified experimental error, V (r) the variation in radiographic technic, and V (t) the variation in the tracing and measuring technic. As an aid to recognition, V (t) may be identified as the average variance within sets of measurements obtained from repeated tracings of the same head plates.

Now coming to the specific problem, at the Burlington Research Centre information has to be collected as economically as possible. Hence it became necessary to consider whether the efficiency of the estimates could best be improved by further refinements of the cephalometric technic, such as duplicating radiograms and tracings, or by increasing the sample sizes. The gains in efficiency which would be obtained in one or the other of these ways had, of course, to be weighed against the cost of producing the duplicate tracings from the same or from duplicate radiograms, and the administrative difficulties and cost of organizing the appointments and maintaining the cooperation of a larger sample of children.

METHOD

Fifteen 3-year-old children constituted the sample for the study, and duplicate sets of radiograms of each child were taken on the same day. From each set of radiograms duplicate tracings were prepared (on different occasions) on acetate paper and were identified as follows:



Using these tracings, the distance between various key points in the skeletal and dental structures were measured and from these measurements 4 sets of data

were produced. Fig. 1 illustrates a measurement on the median sagittal plane which uses a landmark that can be difficult to locate, viz., Bolton Point. This distance, Bolton Point to nasion, is taken from lateral head plates. Fig. 2 shows a horizontal measurement near the periphery of the oblique film representing structures away from the median sagittal plane and near the casette. In

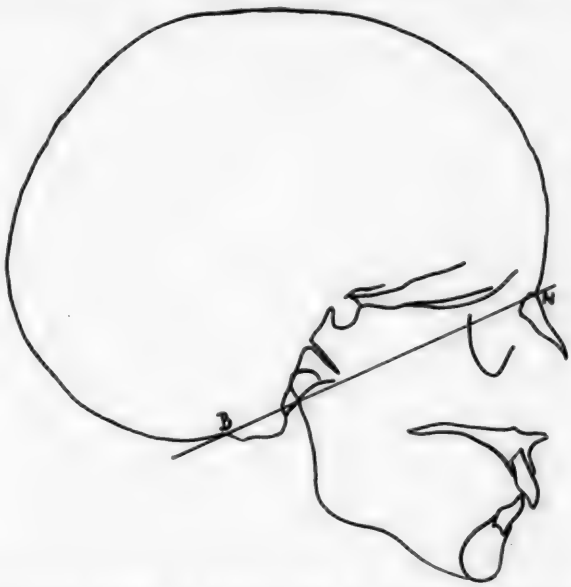


Fig. 1.—Measurement of distance on median plane of lateral head plate from Bolton Point to nasion.

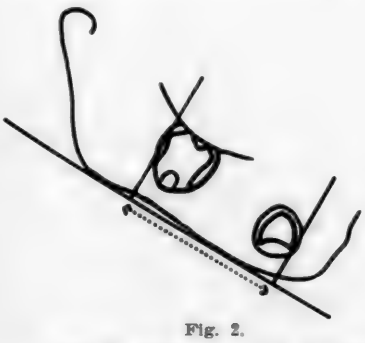


Fig. 2.

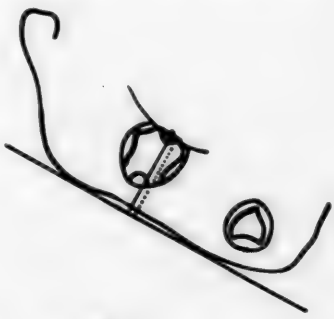


Fig. 3.

Fig. 2.—Measurement of a horizontal distance along the body of the mandible from an oblique radiogram.

Fig. 3.—Measurement of a vertical distance on the body of the mandible from an oblique radiogram.

this case, the landmarks, the distal margin of the first permanent molar, and the mesial border of the cuspid are not difficult to identify. This measurement is called for convenience the "mesio-distal mandibular measurement." Fig. 3 is an example of a short vertical measurement. In making this measurement the

perpendicular distance from the mid-point between the two highest cusps of the first permanent molar to a theoretical plane tangent drawn to the inferior border of the mandible was taken and called molar height.

The estimates of the tracing error variances, $v(t)$, for the 3 distances being considered, were computed with a single degree of freedom from each of the thirty differences between pairs of tracings from the same film.* These estimates were then combined into average estimates based on 30 degrees of freedom. The differences between pairs of tracings from different radiograms reflect both the tracings and the radiographic error, hence each of the sixty variance estimates† represent the sum of the components, $v(t)+v(r)$. As with the tracing variances, these double component variances were combined into average estimates based this time on 60 degrees of freedom. The variance components for radiography alone were then estimated by subtracting the tracing components thus:

$$v(t) + v(r) - v(t) = v(r).$$

The 6 average technical error variances with a 96 per cent confidence range are given in Table I. It is perhaps surprising to notice that the width of these confidence ranges indicates that the technical error variances are not particularly closely estimated and this fact is a caution against drawing anything but broad inferences regarding the relative seriousness of technical error from the different mechanical steps. At the same time, the observed magnitude of the components is of real interest when viewed in the light of the anatomic structures considered and the measurements made.

Using the error variances from Table I, and bearing in mind, as stated above, that these variance estimates are in turn subject to considerable sampling error, it is possible to set down in Table II the approximate limits of improvement theoretically possible by the elimination of all or part of the tracing and/or radiographic error. At the top of this table are the variances for sets of data produced by single tracings and measurements of single radiograms. In the center section are suggested the theoretical (but unobtainable) limits of reduction of the distribution variances which might be achieved if an infinite number of replicas could be averaged to eliminate all technical variation due to radiography and tracing. These were obtained by subtracting the whole variance component of Table I from the distribution variance at the top of Table II. These subtractions were done only to allow the relative importance of the technical error in these specific data to be appreciated and have no other application because in practice the real and technical components of variance are inseparable. At the bottom of Table II are actual reduced distribution variances computed directly from data which were sets of individual averages of the duplicate measurements.

*From each child the 2 tracing variance estimates are $\frac{(a-b)^2}{2}$ and $\frac{(c-d)^2}{2}$.

†From each child the 4 tracing plus radiography variance estimates were $\frac{(a-c)^2}{2}$, $\frac{(a-d)^2}{2}$, $\frac{(b-c)^2}{2}$, $\frac{(b-d)^2}{2}$.

These reductions could have been estimated for these particular data using the error variances of Table I and hence provided some mutual check on the computations.

TABLE I

ANALYSIS OF 3 CEPHALOMETRIC MEASUREMENTS (MM) OF 15 3-YEAR-OLD CHILDREN TO SHOW VARIANCE COMPONENTS ATTRIBUTABLE TO TRACING AND TO RADIOGRAPHIC ERROR

ESTIMATED VARIANCE COMPONENT	BOLTON TO NASION	MESIODISTAL MANDIBULAR MEASUREMENT	MOLAR HEIGHT
v(t)	0.29 (.18 to .54)*	0.059 (.037 to .108)*	0.078 (.049 to .144)*
v(r)	0.25 (.16 to .46)*	0.016 (.010 to .029)*	0.062 (.039 to .114)*

*96 per cent confidence range estimated from χ^2 tables.[3]

TABLE II

THEORETICAL AND PRACTICAL REDUCTION OF TRACING AND RADIOGRAPHIC ERROR AND GAINS IN PRECISION EXPRESSED AS A PERCENTAGE INCREASE IN NUMBER OF SINGLE FILM, SINGLE TRACING OBSERVATIONS NEEDED FOR EQUAL ACCURACY

TECHNICAL SOURCE OF DATA AND VARIANCE COMPONENTS	BOLTON-NASION (MEAN 112.0 MM.)		MESIODISTAL MAND. MEASUREMENT (MEAN 38.4 MM.)		MOLAR HEIGHT (MEAN 17.1 MM.)	
Single Film and Single Tracing	s^2	Relative Sample for Equal Precision Without Duplication	s^2	Relative Sample for Equal Precision Without Duplication	s^2	Relative Sample for Equal Precision Without Duplication
$s^2 = v + v(t) + v(r)$	14.72	100%	3.95	100%	0.534	100%
Theoretical Elimination of:	(s^2)	%	(s^2)	%	(s^2)	%
All Tracing Error $(s^2) = v + \frac{v(t)}{\infty} + v(r)$	14.43	102.0	3.89	101.5	0.456	117.1
All Radiographic Error $(s^2) = v + v(t) + \frac{v(r)}{\infty}$	14.47	101.7	3.94	100.3	0.472	113.2
All Tracing and Radiographic Error $(s^2) = v + \frac{v(t) + v(r)}{\infty}$	14.18	103.8	3.88	101.8	0.394	135.5
By Direct Computation of Average Measurements:	(s_2)	%	(s^2)	%	(s^2)	%
Duplicate Tracing from Single Radiograph $(s^2) = v + \frac{v(t)}{2} + v(r)$	14.57	101.0	3.92	100.8	0.498	107.2
Duplicate Films with Single Tracings $(s^2) = v + \frac{v(t) + v(r)}{2}$	14.44	101.9	3.91	101.0	0.462	115.6
Duplicate Tracings from Duplicate Films $(s^2) = v + \frac{v(t)}{4} + \frac{v(r)}{2}$	14.36	102.5	3.90	101.3	0.445	120.0

In order to understand the practical significance of the reductions in measurement error, the gains in precision in Table II (equal to ratios $\frac{s^2}{(s^2)}$) were

expressed as the percentage increase in numbers of single tracing, single film observations which would yield mean estimates with the same standard errors as those obtained by the duplicate methods. This percentage increase in the required sample is equal to the ratio of the sample distribution variance, s^2, over the particular reduced variance (s^2) which is obtained by computing the average observations.

DISCUSSION

It is evident from Table II that there would be a practical advantage (in money and in time) in improving the reliability of the measurements by making small increases in the number of subjects rather than by duplicating whole sets of tracings or radiograms. As an example, take the most extreme case, the measurement of molar height: the cost of producing a second set of 15 radiograms, plus 3 additional sets of 15 tracings, might be 50 times the cost of producing single tracings from single radiograms of 3 more subjects.

Another advantage to be derived from data which are based on single tracings of single films is that they ensure the correct interpretation of ranges which might be used in clinical diagnosis. Thus, whereas estimates of parameters based on duplicate measurements would be slightly more precise, the ranges for duplicate measurements would underestimate the ranges for single measurements usually available clinically.

It is felt that by relating the magnitude of the technical error variance to the magnitude of the distribution variance, or by expressing both in actual units, one is choosing the clearest way to interpret the practical seriousness of the technical error which is encountered in producing cephalometric data. The tracing errors, and the radiographic errors from the measurements explored here were 2.0, 1.5, 14.6 per cent, and 1.7, 0.5, 11.5 per cent of the distribution variances, for Bolton Point to nasion, mesiodistal mandibular measurement, and molar height, respectively.

Finally, it is worth noting the difference between the foregoing computations of random variation about the assumed average technical error of zero, and the procedure for detecting a real average difference (referred to in the introduction as bias) between two sets of data derived, for example, from head plates of the same subjects but produced on different equipment. If the pairing scheme is known, the standard error for the observed average difference is obtained from the formula $\sqrt{\frac{\Sigma d^2-(\Sigma d)^2/N}{N(N-1)}}$, where d's are the individual differences between respective pairs, N is the number of pairs and, N-1 is the degrees of freedom for testing the hypothesis that $\bar{d}$ is greater than zero.

SUMMARY

Today much emphasis is being placed on the use of cephalometric analysis as a means of clarifying problems of growth and development of the skeleton. The present study has attempted to define the efficiency and reliability of this method

for studying anatomic variations. The method was to compute the error variances involved in the radiographic and tracing technic with data derived from duplicate radiograms and duplicate tracings of 15 3-year-old children. The results showed that, as applied by the present investigators, the technical process is highly refined and that no practical improvement is needed. It was found that by far the greatest source of distribution variation is due to the real difference between children and that from the point of view of economical production of data for creating norms and permitting comparisons, the best experimental procedure is to use a sufficient number of children rather than to attempt to reduce technical error further by duplicating measurements.

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THE EFFECT OF FEEDING CALCIUM PHOSPHATE SALTS WITH DIFFERENT SOLUBILITIES ON DENTAL CARIES, THE COMPOSITION OF THE SALIVA, AND THE FEMURS OF RATS

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PRESENT research suggests that partial control of human dental caries is possible by two completely different mechanisms: (1) developmentally, and, (2) post-developmentally. As an illustration of the former, one may cite the reduction in caries afforded by the fluoride ion when incorporated into the developing tooth. The caries reduction resulting from the topical application of fluorides to the erupted tooth is an example of the post-developmental effect. However, when considering the maximum benefit as well as the duration of effectiveness derived individually from each of the two suggested anticariogenic mechanisms, it is clear that the effects of the developmental factor are much more significant. It appears that our best hope for a pronounced caries reduction will result from developing a sound tooth instead of attempting to make a developmentally unsound tooth less prone to decay after eruption. The single disadvantage to this concept is that it carries with it the necessity of cooperation of every parent and child in making constantly available to the young child the anticariogenic agent. Since this is impossible, even with such simple procedures as communal fluoridation, since all communities will never fluoridate, the practical aspect of caries control must be such that the maximum use is made of post-developmental cariogenic factors. One such possible post-developmental factor deserving consideration is the modification of the composition of saliva which, if properly understood, would result in less dental caries. The purpose of this study is then twofold, in providing answers to the following questions: (1) can the salivary calcium-phosphorus content of rat saliva be modified by feeding calcium phosphate salts and, (2) what is the effect of different inorganic calcium phosphate salts with varying solubilities on the dental caries experience in the rat? This latter point is of interest, since unpublished evidence from our laboratories suggests that, if a soluble calcium-phosphorus salt is ingested along with the daily dietary constituents, this soluble salt is preferentially solubilized instead of the soluble constituents of the tooth. Moreover, this study will make it possible to correlate changes in salivary calcium-phosphorus content with the dietary calcium-phosphorus content and the dental caries experience in the rat.

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EXPERIMENTAL

A total of 150 weanling Sprague-Dawley strain rats were divided into 5 equal groups according to sex and initial body weight. Of these, 135 rats finished the experiment. Group I animals were the control, and received a cariogenic diet composed of (in per cent) yellow corn grits, 52.7; ground yellow corn, 11.3; whole milk powder, 30.0; alfalfa, 4.8; iodized sodium chloride, 1.0; and irradiated yeast, 0.2. The animals in Groups II through V received the same diet as those in Group I except that either 1 per cent or 5 per cent dicalciumphosphate dihydrate or calcium pyrophosphate was substituted for a respective amount of corn grits (see Table I). Food and fluoride-low drinking water (0.05 μg F^-/ml.) were provided ad libitum. The rats were housed in pairs in raised screen cages in an air-conditioned room.

After 120 days, 10 animals of each group were anesthetized with sodium Nembutal (0.7 ml. of a 0.5 per cent stock solution of sodium Nembutal per 100 Gm. body weight was injected intraperitoneally), and pilocarpine hydrochloride (10 mg. per 100 Gm. body weight) was injected intraperitoneally to increase salivary flow so that salivary calcium and phosphorus could be determined. This was accomplished by thorough rinsing of the oral cavity with distilled water and collecting 2 ml. of saliva from each rat. Eight milliliters of 10 per cent trichloroacetic acid were added to each 2 ml. saliva, and after centrifuging, 5 ml. of the trichloroacetic acid-saliva solution were used for the calcium and 3 ml. for phosphorus determination. Calcium was analyzed by oxalate precipitation and subsequent titration with potassium permanganate.[1] Phosphorus was determined by the method of Fiske and Subbarow.[2] Since the saliva-trichloroacetic solution was turbid, it was necessary to precipitate the colloidal, dissolved salivary proteins by adding 1 ml. of a 2 per cent aqueous phosphorus-free bovine gamma globulin solution to each 3 ml. of the trichloroacetic acid-saliva solution. The calcium and phosphorus content of each of the 5 diets was also determined by ashing 30.0 Gm. of the various diets at 550° C. Aliquots of 30 mg. of ash were dissolved in 0.5 ml. of concentrated hydrochloric acid in a 50 ml. volumetric flask. One milliliter aliquots were used for calcium and phosphorus determinations as described above.[1, 2]

All of the animals were sacrificed by ether and the heads removed for caries evaluation as previously described.[3] Calcium and phosphorus were determined in the femurs of each group.

RESULTS AND DISCUSSION

The data on the caries experience and salivary calcium and phosphorus content of the saliva are found in Table I, and the calcium and phosphorus content of the diets and the femurs are shown in Table II.

The weight gain of the animals in Groups II through V was normal and comparable to that of the control (Group I) throughout the experimental period.

TABLE I

SALIVARY CA AND P LEVELS AND DENTAL CARIES EXPERIENCE IN RATS RECEIVING DICALCIUMPHOSPHATE AND CALCIUM PYROPHOSPHATE IN THEIR DIETS

GROUP	DIET	NO. OF RATS	DENTAL CARIES EXPERIENCE, MEAN NO. OF LESIONS	SALIVARY Ca AND P LEVEL				
				NO. OF SAMPLES	MEAN Ca		MEAN P	
					MG. %	RANGE	MG. %	RANGE
I	Stock corn diet (Control)	25	8.6 ± 0.5*	10	4.4 ± 0.3*	2.9-7.2	1.1 ± 0.1*	0.7-1.8
II	1% $CaHPO_4 \cdot 2H_2O$	27	7.2 ± 0.7	10	4.4 ± 0.6	1.4-8.9	1.7 ± 0.2	1.0-2.2
III	5% $CaHPO_4 \cdot 2H_2O$	27	8.4 ± 0.5	10	3.7 ± 0.4	1.7-6.2	2.0 ± 0.2	1.0-3.7
IV	1% $Ca_2P_2O_7$	26	8.6 ± 0.5	10	4.7 ± 0.9	2.6-9.4	2.2 ± 0.1	1.5-2.8
V	5% $Ca_2P_2O_7$	30	8.6 ± 0.5	10	2.6 ± 0.4	0.3-5.5	1.6 ± 0.2	0.8-3.2

*Standard deviation.

TABLE II
CALCIUM AND PHOSPHORUS ANALYSIS OF DIETS AND FEMURS OF EXPERIMENTAL ANIMALS

GROUP	DIET	DIET ANALYSIS			FEMUR ANALYSIS*				
		P (GM./100 GM. OF FOOD)	Ca (GM./100 GM. OF FOOD)	RATIO Ca:P	% P IN ASH	P†	% Ca IN ASH	P†	RATIO Ca:P
I	Stock corn diet (Control)	0.31	0.32	1.03	17.2 ± 0.1‡	–	32.8 ± 0.3‡	–	2.02
II	1% $CaHPO_4 \cdot 2H_2O$	0.42	0.56	1.33	17.5 ± 0.1	0.1	36.5 ± 0.7	0.1	2.08
III	5% $CaHPO_4 \cdot 2H_2O$	1.10	1.62	1.47	17.5 ± 0.1	0.05	37.5 ± 0.9	0.02	2.14
IV	1% $Ca_2P_2O_7$	0.48	0.68	1.42	17.6 ± 0.2	0.05	36.8 ± 0.6	0.02	2.08
V	5% $Ca_2P_2O_7$	1.10	1.88	1.70	17.7 ± 0.1	0.01	37.3 ± 0.3	0.01	2.11

*The values represent mean values of 6 femurs per group.
†Probability, based on "t" test.
‡Standard deviation.

The dental caries data indicate no differences in any of the groups. Similarly the calcium and phosphate content of the saliva in each of the experimental groups indicate no significant differences, although there is a tendency for less salivary calcium in the group receiving 5 per cent calcium pyrophosphate, and more phosphorus in each of the calcium phosphate supplemented groups when compared to the controls. The magnitude of change in the phosphorus content is not significantly great to modify the caries experience, or it may well be, however, that the phosphorus content of the saliva is not related to the caries experience in the rat. In comparison with the chemical composition of human saliva it is interesting to note that the calcium range of 4 to 8 mg. per cent in human saliva corresponds with the range found in saliva of rats, while the amount of phosphorus in rat saliva is much lower than found in the human saliva, which is 10 to 25 mg. per cent.

The calcium and phosphorus content of the femurs are seen in Table II, and show that there is more calcium and phosphorus in each of the 4 groups which received the calcium phosphate supplements. It is of particular interest to note that the insoluble calcium pyrophosphate increased the skeletal calcium and phosphorus content as much as the soluble dicalciumphosphate. While there is significantly more calcium and phosphorus in the femurs in the animals receiving 5 per cent calcium pyrophosphate, there is numerically less calcium and phosphorus in the saliva. The physiologic significance of this is not known at the present time.

CONCLUSIONS

The administration of either 1 or 5 per cent dicalciumphosphate dihydrate or calcium pyrophosphate as a constituent of the daily diet of rats did not reduce their dental caries experience or significantly modify the calcium content of the saliva. However, the feeding of the 2 calcium-phosphate salts did increase the phosphorus content of the saliva, as well as significantly increasing the calcium and phosphorus content of the femurs, when evaluated on a percentage of ash-weight basis.

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EXPERIMENTAL CARIES WITH HUMAN FOODS

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SEVERAL types of diets have been used in the study of dental caries. One of the more common diets[1, 2] used contains ingredients, such as coarse corn meal, which cause fracturing associated with caries production. Another general type of diet[3, 4, 5] is largely synthetic and contains large amounts of sucrose. A third dietary type[6, 7, 8] contains human foods with smaller amounts of sucrose. In this latter group, a limited variety of human foods are used, which are dehydrated, ground in a mill, and fed to the animals. As generally recognized, particle size, moisture content, and physical consistency of the food may affect the amount of caries produced.

The caries-susceptible albino rats used in these studies were descendents of 8 animals obtained from the Naval Medical Research Institute at Bethesda, Maryland.

The diets employed represent the average American diet and modifications thereof and are composed of human foods as customarily prepared and eaten. The foods were thoroughly mixed to make a uniform moist mass of rations which were fed to the animals ad libitum.

MATERIALS AND METHODS

Basis of Formulating the Diets.—The percentage composition of the diet is based on the table of U. S. food supply as computed by Callison and co-workers.[9] This is in close agreement with the findings of Hardinge and Stare[10] who made a study of food consumption in California.

From the basic diet thus computed, the cereals and desserts were removed and were substituted on an equicaloric basis with whole grain cereals (in place of refined cereals), and fruits or fruits and vegetables (in place of desserts). The latter presented the problem of replacing concentrated foods high in calories with foods low in calories but high in fiber and water.

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In the consumption of the individual foods or food groups in both the basic ration and the accessory groups, namely cereals, fruits, vegetables, and desserts, the amount of the individual constituents were proportioned according to the average American per capita consumption.[11] For practical reasons, only representative foods, used in the largest amounts in each food group, were proportioned according to their popularity in this country as follows: meat—beef and pork; dairy products—milk, cheese, eggs; potatoes—white; vegetables other than potatoes—carrots, cabbage; fruits—apple, orange, and banana; cereals—refined wheat and corn, whole wheat and corn; desserts—cake and pie; fats—dairy butter, lard, hydrogenated shortening. The composition of the various foods groups is shown in Table I.

TABLE I
COMPOSITION OF FOOD GROUPS

GROUP	FOODS	WEIGHT (GM.)	GROUP	FOODS	WEIGHT (GM.)
A	Basic Ration		C	Whole Grain Cereals	
	Canned beef	61		Whole wheat bread	824
	Canned pork	51		Unbolted corn meal	176
	Canned potatoes	120	D	Desserts	
	Whole milk	610		Cake, plain white, iced	502
	Canned carrots	83		Pie, lemon meringue	498
	Raw cabbage	92	E	Fruits	
	Canned kidney beans	100		Apple, raw	394
	Eggs	54		Orange	444
	Raw apple	94		Banana, raw	162
	Orange	89	F	Fruits and Vegetables	
	Canned peaches	41		Carrots, raw	122
	Butter	23		Cabbage, raw	270
	Crisco	19		Apple, raw	240
	Lard	20		Orange	270
B	Refined Cereals			Banana, raw	98
	White enriched bread	807			
	Refined corn meal	193			

The diets consisted of many of the combinations of these groups, as shown in Table II. The quantity of food mixture from individual groups combined to make up the several diets, was so proportioned as to keep the diets isocaloric, as well as to provide nearly equal intakes of carbohydrate and protein.

TABLE II
COMPOSITION OF DIETS

DIET	FOOD GROUPS	WEIGHT (GM.)	DIET	FOOD GROUPS	WEIGHT (GM.)
I	A	784	V	A	504
	B	95		C	69
	D	121		E	427
II	A	508	VI	A	459
	B	62		C	63
	E	430		F	478
III	A	462	VII	A	892
	B	56		B	108
	F	482	VIII	A	880
IV	A	774		C	120
	C	106			
	D	120			

For purposes of comparison a modification of the synthetic diet of Sognnaes[5] was fed to Group IX as a control. This diet had the following composition: casein, 24; sucrose, 65; salts U.S.P. XIV, 4; dehydrated liver, 4; vitamin mix,[2] 2; choline, 0.2; corn oil, 5.

An analysis of the various diets is given in Table III.

TABLE III
FOOD ANALYSIS OF VARIOUS DIETS

DIET	WEIGHT (GM.)	CALORIES	PROTEIN (GM.)	FAT (GM.)	CHO (GM.)	Ca (MG.)	P (MG.)	Fe (MG.)	A* (I. U.)	C (MG.)	B_1 (MG.)	B_2 (MG.)	NIACIN (MG.)
I ABD	1902	3000	86	108	334	1165	1375	10.8	13,300	125	1.68	2.73	9.58
II ABE	2931	3000	85	93	403	1237	1434	14.7	15,400	444	3.09	2.98	12.6
III ABF	3225	3000	91	93	395	1444	1576	17.0	35,700	584	3.53	4.16	13.8
IV ACD	1925	3000	89	108	339	1232	1758	14.1	13,400	125	3.45	2.86	13.6
V ACE	2954	3000	88	93	408	1304	1814	18.9	13,900	444	3.60	3.10	16.6
VI ACF	3248	3000	94	94	400	1511	1956	20.0	35,700	584	4.05	4.29	17.9
VII AB	2228	3000	101	119	289	1336	1600	14.0	17,200	167	2.17	3.31	12.12
VIII AC	2258	3000	105	120	299	1431	2107	17.2	17,600	167	2.84	3.59	17.6

*Includes carotene.

The foods comprising each diet were passed through a Hobart food grinder twice and then thoroughly mixed. To limit further the preferential selection of individual foods within a diet, only enough food was given to last the animals 1 day. The foods were prepared fresh 3 times a week and stored in the refrigerator until served to the animals. To make the foods as uniform as possible throughout the 16 weeks, canned foods were purchased by the case.

One hundred seventeen weanling rats, 21 days of age, were divided by litters into 9 groups of 13 animals each and placed on their respective diets. Similar groups and numbers of animals were employed for successive generations. Ten weeks after weaning, 3 females and 1 male, generally littermates, were selected from each of the 9 diet groups for breeding each generation. The animals remained on their respective diets throughout breeding, gestation, and lactation. At the end of 16 weeks, the animals were sacrificed and the teeth examined with a 20 × dissecting microscope by grinding successive planes parallel to occlusal surface. Individual charts which showed the relationship of the enamel, dentin, and the pulp of each molar were used to record the carious process. The lesions were scored by the method of Shaw and co-workers.[3]

The nutritional status of the animals from generation to generation was quantitatively evaluated by noting weaning weights, weekly gain, total weight, length of mandible (measured from the distal surface of the third molar to the tip of the incisor), ability to reproduce, and general physical appearance. Considerable difficulty was encountered in breeding the third generation of animals on Diet VI. Twenty-one days following birth, the young weighed an average of 18 grams. Since this weight was too small for weaning, the animals were left with their mothers for an additional 2 weeks to attain sufficient weight.

FINDINGS

The caries score and nutritional status were determined for each group of animals through successive generations. This information is summarized in Table IV.

TABLE IV

THE EFFECT OF VARIOUS DIETS ON CARIES SCORE AND NUTRITIONAL STATUS THROUGH SUCCESSIVE GENERATIONS

TYPE OF DIET	NUMBER OF ANIMALS	GENERATION	CARIES SCORE	STANDARD ERROR OF THE MEAN	NUTRITIONAL STATUS					
					WEANING WEIGHT	AVERAGE WEEKLY GAIN	TEN-WEEK WEIGHT	REPETITION OF BLEEDING	LENGTH OF MANDIBLE	GENERAL APPEARANCE
I	13	1	13.6	3.88	56	20.2	228		*	
ABD	13	2	23.0	4.16	43	21.0	226	1	19.51	
	13	3	23.2	2.64	35	17.5	204	2	19.13	
II	13	1	11.8	2.76	53	18.0	201		*	
ABE	13	2	26.5	5.20	26	14.2	152		19.15	
	13	3	13.3	2.36	39	15.4	184		19.48	
III	13	1	7.0	1.42	54	18.0	202		*	
ABF	13	2	8.6	1.51	37	15.6	153		19.37	
	13	3	14.1	2.66	27	11.8	147		19.51	
IV	13	1	1.3	0.84	57	23.0	228		*	Poor
ACD	13	2	16.3	3.54	34	19.0	199		19.23	
	13	3	28.8	5.65	30	18.0	216		19.97	
V	13	1	2.33	0.98	58	20.0	215		*	
ACE	13	2	13.8	2.53	31	15.6	160		18.62	
	13	3	10.5	1.76	39	16.8	195		19.27	
VI	13	1	5.5	1.47	55	15.8	192		*	
ACF	13	2	27.7	4.76	38	13.6	180	2	18.90	
	13	3	37.5	3.58	18	11.0	151	3	18.63	
VII	13	1	1.5	0.62	55	29.0	232		*	
AB	13	2	1.1	0.40	52	23.8	228		19.64	
	13	3	7.7	2.18	48	23.6	254		20.16	
VIII	13	1	0.23	0.16	56	26.2	255		*	Good
AC	13	2	1.2	0.55	38	21.6	204		19.39	
	13	3	2.8	0.99	45	21.6	236		19.79	
IX	13	1	6.3	2.86	55	26.4	245		*	
SYN.	13	2	4.2	1.02	49	30.2	260		19.89	
	13	3	10.5	2.68	64	25.2	256		19.96	

*First generation mandibles not available.

TABLE V
TABLE OF CARIES INDEX AND NUTRITIONAL INDEX AS DETERMINED FROM PHYSICAL FINDINGS

GROUP	GENERATION	CARIES INDEX (C)	WEANING WEIGHT	AVERAGE WEEKLY GAIN	10-WEEK WEIGHT	BREEDING	MANDIBLE LENGTH	GENERAL APPEARANCE	NUTRITIONAL INDEX (X)
I	1	64.1	82.6	47.9	71.7	100	*	25	65.4
	2	38.9	54.3	52.1	69.9	67	57.9	25	54.3
	3	38.4	37.0	33.9	50.0	33	33.2	25	35.4
II	1	69.0	76.1	36.5	47.8	100	*	25	57.1
	2	29.5	17.4	16.7	4.4	100	34.4	25	33.0
	3	64.9	45.7	22.9	32.7	100	55.8	25	47.1
III	1	81.8	78.3	36.5	48.7	100	*	25	57.7
	2	77.5	43.3	24.0	5.3	100	48.7	25	40.7
	3	62.8	19.6	4.2	00.0	100	57.8	25	34.5
IV	1	97.1	84.8	62.5	71.7	100	*	25	68.8
	2	56.9	34.8	41.7	46.0	100	39.6	25	47.7
	3	23.3	26.1	36.5	61.1	100	22.7	25	45.3
V	1	94.4	87.0	46.9	60.2	100	*	25	63.8
	2	63.9	28.3	24.0	11.5	100	0.0	25	31.5
	3	72.4	45.7	30.2	42.5	100	42.2	25	47.6
VI	1	85.9	80.4	25.0	39.8	100	*	25	54.0
	2	26.3	43.5	13.5	29.2	33	18.2	25	27.0
	3	00.0	0.0	0.0	3.5	00	0.7	25	4.9
VII	1	96.9	80.4	93.8	75.2	100	*	75	84.9
	2	97.7	73.9	66.7	71.7	100	66.2	75	75.6
	3	80.0	65.2	65.6	94.7	100	100.0	75	83.5
VIII	1	100.0	82.6	79.6	95.6	100	*	75	86.6
	2	97.4	43.5	55.2	50.0	100	50.0	75	62.0
	3	93.1	58.7	55.2	78.8	100	76.0	75	73.9
IX	1	83.7	80.1	80.2	86.7	100	*	75	84.5
	2	89.3	67.4	100	100	100	82.5	75	87.5
	3	72.4	100	74.0	96.5	100	87.0	75	88.8
Mean		68.8							57.1

*First generation mandible measurement not available.

In order to evaluate the nutritional state and the incidence of caries, the various measurements used to define nutritional state were scored on a scale of 1 to 100. An average for the nutritional state of each group was determined. For purposes of convenience this has been called the "nutritional index." Similiarly the caries score was rated in order to obtain what has been called the "caries index." These are presented in Table V.

As seen in Table VI the differences in length of mandibles in the third generation of animals are small. The mean length of mandibles of each group was statistically compared with the mean length of the group having the shortest mandibles, namely Group VI. As can be seen from Table VI the variations in mandibular lengths are highly significant.

TABLE VI
STATISTICAL ANALYSIS OF MANDIBLE MEASUREMENTS* OF THIRD GENERATION ON VARIOUS DIETS

			Significance
Diet I	$X = 19.13$ $\sigma = 0.6$ $SE = 0.172$	$t = 2.31$	.04
Diet II	$X = 19.48$ $\sigma = 0.435$ $SE = 0.125$	$t = 4.61$	.01
Diet III	$X = 11.51$ $\sigma = 0.624$ $SE = 0.208$	$t = 3.53$	.01
Diet IV	$X = 18.97$ $\sigma = 0.787$ $SE = 0.227$	$t = 1.21$	>.05
Diet V	$X = 19.27$ $\sigma = 0.485$ $SE = 0.140$	$t = 3.33$	.01
Diet VI	$X = 18.63$ $\sigma = 0.469$ $SE = 0.132$		
Diet VII	$X = 20.16$ $\sigma = 0.538$ $SE = 0.156$	$t = 7.50$	.001
Diet VIII	$X = 19.79$ $\sigma = 0.705$ $SE = 0.213$	$t = 4.64$	.01
Diet IX	$X = 19.96$ $\sigma = 0.608$ $SE = 0.180$	$t = 5.96$	.01

*Mean mandible length of respective groups compared with mean mandible length of group with shortest mandible, *i.e.*, Diet VI.

DISCUSSION

A detailed analysis of the nutrient content of the various diets used in this study was presented. The long-term effect of these diets is of special significance since minor differences in nutrient content will tend to be either compensated for or magnified through successive generations. An attempt has

been made to compare these differences by means of the nutritional and caries indices. By formulating a caries index "C" and a nutritional index "N," regression lines were derived and the coefficient of correlation calculated for these indices (Fig. 1). The regression of "C" on "N" is 0.965. The coefficient of correlation is 0.78 ± 0.05. If the nutritional index is known, the most probable

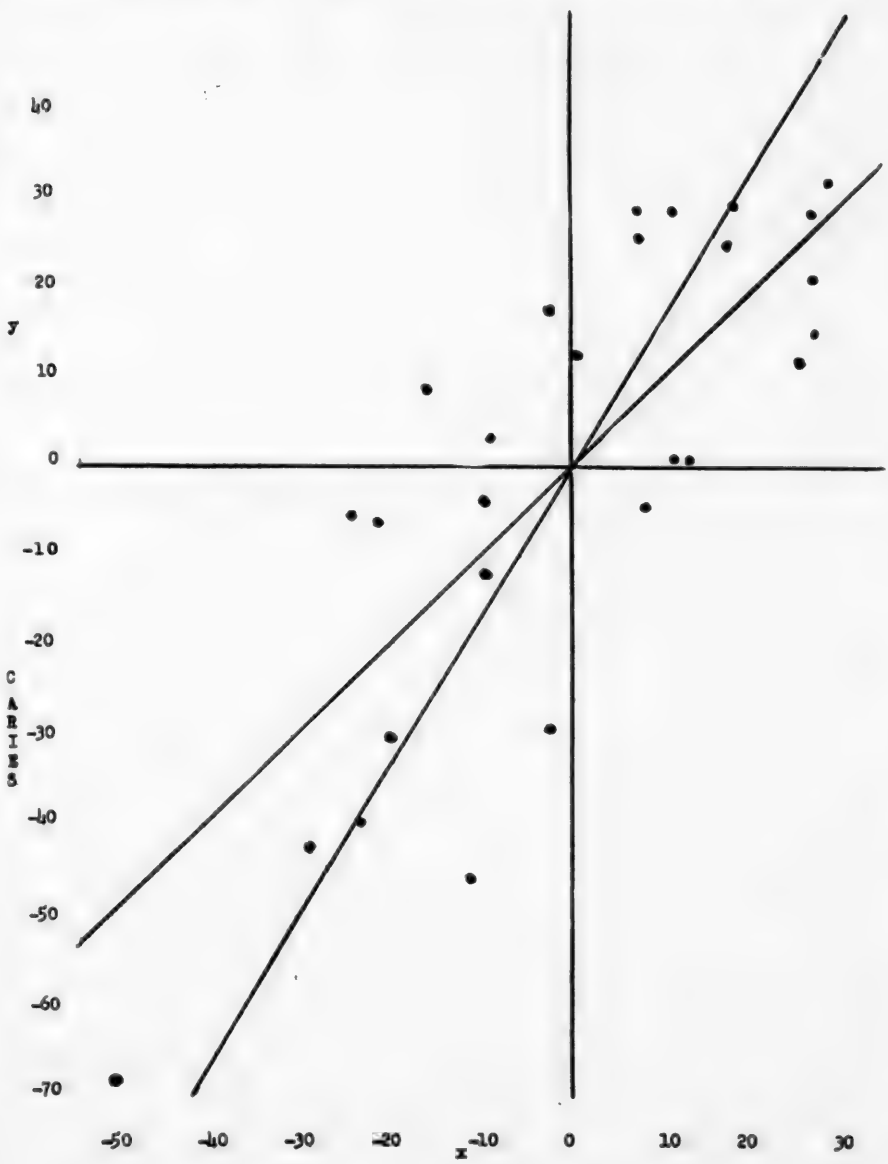

Fig. 1.

caries index may be calculated from the following formula:

I_c = Mean Caries Index + most probable deviation from the mean ± the probable error of the Index
= 68.8 − .965 (I_n—57.1) ± 11.5
= .965 I_n + 13.7 ± 11.5.

The statistical evidence indicates a high inverse correlation between general nutritive status and caries incidence.

The nutritional state of all animals at the beginning of the first generation was uniformly high. It was not until the beginning of the second generation that noticeable differences in weaning weights occurred. Groups I and VI did not reproduce as well as did the other groups.

In Groups I, III, IV, and VI there was a steady decline in general nutritional status in the second and third generations. This was most noticeable in Group VI. This physical deterioration was associated with a concomitant increase in caries.

The nutritional well-being of Groups II and V degenerated between the first to the second generation with a corresponding increase in caries. It would appear that these two groups adapted to their dietaries between the second and third generations with an improvement in their nutritional state associated with a decline in caries incidence.

The general physical well-being of Groups VII and VIII remained high and did not significantly vary during the 3 generations studied. Throughout this period, the caries incidence varied but little and remained low. In Group IX, where the standard cariogenic diet was employed for comparative purposes, a constant and relatively high state of nutrition was maintained through successive generations. Again this constancy in physical well-being was accompanied by only minor fluctuations in caries incidence.

CONCLUSION

A critical analysis of the data obtained from 3 generations of rats fed on 8 different combinations of human foods is presented. The nutritional status and physical well-being of all animals showed an inverse correlation with caries incidence. It would appear that with these diets, good nutritional status is associated with a low caries incidence while nutritional deterioration is accompanied by an increase in caries.

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THE EFFECT OF PYRIDOXINE AND INJECTED CARBOHYDRATE ON INCIDENCE OF CARIES, DENTINAL CIRCULATION RELATED TO DIET

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THE etiology of dental caries is still not fully understood. Studies by Orland and the Lobund group[1] with germ-free animals indicate that bacteria are essential in this process, but the mere presence of bacteria does not guarantee caries. The necessity of a fermentable carbohydrate as a substrate for bacterial activity was demonstrated by Kite, Shaw, and Sognnaes[2] in tube-feeding experiments, but there appears to be no consistent correlation between the amount of carbohydrates passing through the oral cavity and the degree of caries present. From the available evidence, the production of caries appears dependent on some additional etiological factor or factors. It is probable that these factors condition the tooth and make it susceptible to the carious process. It is reasonable to suspect that interference with the metabolic cycle contributes to this group of additional etiological factors.

In the experimental study of caries by the authors, 8 combinations of human foods which were utilized in this laboratory have been previously outlined.[3] The ninth diet was a synthetic diet commonly used for the production of caries.[4] As previously reported, a critical analysis of the data obtained from 3 generations of rats fed on 8 combinations of human foods and the synthetic diet revealed that the nutritional state and physical well-being of the animals were directly correlated with the incidence of caries. In general, a good nutritional state was associated with a low incidence of caries while nutritional deterioration was accompanied by an increase in the disease.

EXPERIMENTAL

A dual experimental approach to the problem was made in an attempt to determine, in the posteruptive period, whether cariogenic or noncariogenic diets influenced the "circulation" in the dentin, or whether the caries process was affected by varying quantitatively specific nutrients in the diet.

The extent to which the dentin is able to concentrate trypan blue was employed as a criterion of "circulation" within the dentin. The concentration of

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the color of dye in the tooth varies from barely detectable to distinctly blue. This is most marked in young animals. However, older animals will also exhibit this color deposition but at a somewhat slower rate. A short-term experiment was performed using 35-day-old Purina-fed* rats. All animals were injected with 0.5 ml. of 4 per cent trypan blue intraperitoneally and sacrificed after 5 minutes to 48 hours. The dentin of rats sacrificed 10 minutes after injection of dye showed a slight grayish-blue tinge. The stain continued to increase in density for 48 hours at which time it was distinctly blue.

A long-term experiment was carried out in which littermaters were divided into two groups at weaning. One group was placed on Purina, the other on the synthetic diet previously described. Into all animals, 0.5 ml. of 2 per cent trypan blue was injected intraperitioneally once a week for 3 weeks. At 25 days of age, gold crowns were placed on the lower right first molars of 3 rats in each group. After 12 weeks all animals were sacrificed and their teeth examined for concentration of the dye. The teeth of the animals on the Purina were significantly darker than those on the cariogenic diet. No color difference was observed between comparable crowned and uncrowned teeth in the same mouth.

A microscopic examination of the secondary dentin of the animals on the cariogenic diet showed wavy concentric rings, while those on Purina did not exhibit this formation. Subsequently, a similar examination of 35 rats on the above diets but not receiving the trypan blue also exhibited this configuration of the dentin. These findings are shown in Figs. 1 and 2.

To evaluate the effect on caries production of quantitatively varying specific nutrients, the following experiments were conducted. Thirty 21-day-old rats were divided into 2 equal groups by litters. One group was placed on Diet I[3] (representative foods of average American diet) while the other was fed the same diet supplemented with pyridoxine to the extent of 16 mg. per kilogram of rations. All animals were sacrificed at the end of 13 weeks and the molars examined for caries by the method of Shaw.[5] The results of this study are shown in Table I.

TABLE I
EFFECT ON CARIES OF VITAMIN B_6 ADDED TO A DIET COMPOSED OF TYPICAL AMERICAN FOODS

GROUP	DIET	NUMBER OF ANIMALS	NUBMER OF CARIES-FREE ANIMALS	SCORE	STANDARD ERROR OF THE MEAN	P VALUE
1	Diet 1	15	1	8.8	1.98	
11	Diet 1 plus B_6	15	4	4.0	0.89	0.03

Twenty animals were divided by litters into two groups at 21 days of age. All animals were fed Diet VII[3] (average American diet minus desserts). To one group a mixture of equal parts of glucose and fructose was administered intraperitoneally in a dosage of 1 mg. per gram of body weight 3 times a day. This

*Purina Laboratory Chow, Ralston Purina Co., St. Louis 2, Mo.

dose was selected to parallel the intake of sugar which is generally consumed by the average adolescent American, an amount which approximates 20 per cent of his total calories. After 13 weeks, the animals were sacrificed and the molars examined as previously mentioned. Table II summarizes the results of this part of the study.

Fig. 1.

Fig. 2.

Fig. 1.—Concentric rings in secondary dentin of animals on cariogenic diet. *E*—enamel; *D*—dentin; *C*—concentric rings in secondary dentin.

Fig. 2.—Absence of concentric rings in secondary dentin of animals fed on Purina. *E*—enamel; *D*—dentin; *C*—secondary dentin.

TABLE II
DIET VII PLUS 20 PER CENT GLUCOSE AND FRUCTOSE INJECTIONS GIVEN POSTERUPTIVELY

NUMBER OF ANIMALS	DIET	SCORE	STANDARD ERROR OF THE MEAN	P VALUE
10	Diet VII	3.8	0.95	
10	Diet VII plus glucose and fructose (1 mg./Gm.)	10.9	2.60	0.01

DISCUSSION

The marked difference in the ability of dentin to concentrate trypan blue in animals on the noncariogenic and cariogenic diet would strongly suggest that the "circulation" differs in caries-resistant and caries-susceptible teeth. This postulate is further strengthened by the structural differences, that is, the ring formations in the secondary dentin of animals on the cariogenic diet which are not present in the dentin of animals fed Purina.

The results obtained from supplementing the average American diet with pyridoxine confirm the work of Rinehart and Greenberg[6] who demonstrated the role of this nutrient in the reduction of caries in experimental animals.

The bypassing of the oral cavity by parenterally administered monosaccharides (glucose and fructose) indicates that their cariogenic effect results from systemic action. It would thus appear that carbohydrates may increase the production of caries by both the well-established local action and the currently demonstrated systemic effect.

In a previously reported paper,[3] it was demonstrated that the nutritional status as reflected by physical weights and measurements was found to be closely correlated with the incidence of caries. In general, as the animals deteriorated from the first through the third generation, there was also a corresponding deterioration of the teeth. In two groups there was a deterioration from the first to the second generation which was followed by a significant improvement from the second to the third generation. The teeth in these groups paralleled the nutritional state, poor teeth in the second generation and good teeth in the first and third generations. Those groups in which the teeth remained uniformly good through the 3 generations were found to be in a uniformly good physical state.

From a review of the literature, it is clear that evidence indicates that the production of caries in the posteruptive period is affected by factors which may influence circulation, nutrition, and metabolism.

Brewer, Muhler, and Fischer[7] demonstrated that the decreased vascularity resulting from ligation of the major vessels to the teeth caused a significant increase in caries. The chewing of hard substances was found by Neumann, Lefkowitz, and DiSalvo[8] to reduce the incidence of caries. This might be explained by the resultant stimulation of circulation.

Several factors affecting both metabolism and caries have been reported. As already mentioned, the incidence of caries in hamsters and monkeys can be

influenced by the concentration of pryidoxine in the diet.[6] Bavetta and McClure,[9] McClure,[10] and others found that L-lysine deficiencies increased the incidence of caries. X-ray therapy was shown by Schlack and Ellinger[11] to augment the caries process in experimental animals. Del Regato[12] has also observed this clinically. Whether these latter observations are a result of disturbed circulation or metabolism has not been resolved. Bixler, Muhler, and Shafer[13, 14] found androgens increased caries in rats. Workers in this group[15, 16] also demonstrated that by altering the concentration of circulating thyroxin the caries process could be retarded or augmented. Mitchell and Johnson[17] observed that the administration of acetazoleamide (Diamox) significantly increased caries in hamsters. Whether the role of this componud as a carbonic anhydrase inhibitor is implicated in its cariogenic action has not been established.

SUMMARY AND CONCLUSIONS

The "circulation" within the dentin and the effect of quantitatively varying specific nutrients was investigated in caries-susceptible rats. From the evidence presented, it would appear that the "circulation" in the dentin is perceptibly different in animals fed cariogenic and in those fed noncariogenic diets. Structural differences in the secondary dentin were also observed in these animals. Parenterally administered glucose and fructose significantly increased the incidence of caries.

It would appear that, besides the bacterial flora in the mouth and the local presence of fermentable carbohydrates, a variety of systemic factors enumerated above also contribute to the caries process. It would be reasonable to assume that the theory long held that caries is a purely local problem is now no longer tenable. The work of many investigators, cited above, together with the findings reported in this paper, favors the concept of a disturbance in the metabolism of the host which predisposes to the development of caries by lowering the resistance of the tooth.

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A TECHNIQUE FOR THE STUDY OF THE INTERNAL STRUCTURE OF CALCIFIED TISSUES

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THE investigations of Williams and Irvine[1] showed that extraction of organic material from bone with ethylenediamine (ED) was superior to ashing with KOH-glycol. Since the anorganic skeleton prepared in the former manner appeared to be intact, the method was utilized by them in studies of trabecular stress patterns. They also noted that less alteration of crystalline structure resulted from preparation of the anorganic bone from ED than from glycol-ashing methods. Peckham and Losee[2] compared the efficiency of ED and KOH-glycol extractions of dentin and found that there was a negligible loss of mineral content with the former method in contrast to an appreciable amount with the latter. This is believed to be due to the relatively low temperature (118° C.) used in removing the organic fractions with ED in a modified Soxhlet extractor. Because ethylenediamine is readily soluble in water, it can easily be washed out of the anorganic matrix after extraction. Losee[3] utilized ED extraction to study the microscopic pathways in enamel and demonstrated the presence of micro-lamellae in human enamel. It was the aim of the present investigation to utilize this improved method of extraction of organic components of calcified tissues in developing a method of studying the internal structure of these tissues.

METHODS AND MATERIALS

Fresh samples of bone and teeth were placed immediately in fritted disk, Pyrex extraction thimbles. The thimbles then were placed in modified Soxhlet extractors (Losee[4]) and the samples were extracted at 118° C. with a solution of ethylenediamine, consisting of 80 parts of ED and 20 parts of distilled water. One hundred milliliters of the solution of ethylenediamine was found sufficient to extract the organic material from samples of fresh bone or teeth up to 5 grams in weight. The extraction was carried out in a hood because of the toxicity of the boiling ethylenediamine. The period of extraction was dependent upon the size of the sample. An average of 48 to 72 hours was found to be necessary for a 5-gram sample. The extracted tissues then were washed for 30 minutes in a constantly changing bath of distilled water previously shaken with anorganic bone powder and filtered. After dehydration in a series of ascending alcohols

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and subsequent removal of the alcohol with ethyl ether, one series of anorganic bone was placed in unsaturated polyester resin liquid (molecular weight approximately 2,000) containing a particulate dyestuff* and transferred to a vacuum chamber. The vacuum was maintained for 30 minutes at a level just below the point of ebullition of the liquid plastic. Then the vacuum was reduced, catalyst was added, and the sample cured. The dye was retained on the walls of the vascular channels while clear plastic filtered through the haversian canals and filled the lacunae, canaliculi, and any other small spaces present. The specimens then were decalcified with 0.1 N HCl. After dehydration, a second impregnation with unsaturated polyester resin resulted in an optically clear specimen except for the vascular channels which were effectively outlined throughout the block with the dyestuff of the initial infiltration.

A modification of this double-embedding technic for bone was accomplished by only partially infiltrating the secondary plastic mass into the specimen. In this manner an interface was gained between the cleared portion of the block and that portion containing plastic in only the spaces previously containing organic matter. Thus, at any desired level in the block, the microstructure of the bone could be revealed. (It should be noted that the curing temperature and the amount of catalyst used in both impregnations must be constant to prevent clouding of the otherwise clear plastic.)

In the third series, the extracted teeth were washed, dehydrated in alcohol, and placed in ethyl ether. The specimens then were quickly transferred into a 60-40 mixture of methyl and butyl methacrylate monomers. Since these monomers have small molecular volumes (in the range of 160 cubic Å), impregnation into very small spaces can be achieved. The volume of the monomer was at least 100 times that of the specimen. To ensure complete impregnation, the tissue was allowed to remain in the monomer for 72 hours. The catalyst† then was added and the plastic cured for 72 hours at 60° C. The sample was decalcified, washed with distilled water, and infiltrated with gelatin. The gelatin was introduced by placing the specimen in a freshly prepared 10 per cent solution for 1 week at 60° C., during which time the aqueous solution was allowed to dehydrate to one half its original volume. The gelatin was gelled in the refrigerator and further hardened by immersion in 10 per cent formalin. These specimens were sectioned at approximately 5-10 μ on the freezing microtome. The resulting plastic-gelatin matrix was sufficiently contrasting to permit microscopic study.

OBSERVATIONS AND DISCUSSION

Ethylenediamine-treated cortical bone appears white, porous, and ashlike as seen in Fig. 1. After impregnation with a plastic containing a dyestuff, decalcification and re-impregnation with a clear plastic, the vascular channels become apparent (Fig. 2). In Fig. 3 a higher magnification of Fig. 2 is seen.

*Ward's BPM Opaque Color, Ward's Natural Science Establishment, Inc., Rochester, N. Y.
†Luperco C D B, Lucidol Division of the Novadel Agene Corp., Buffalo, N. Y.

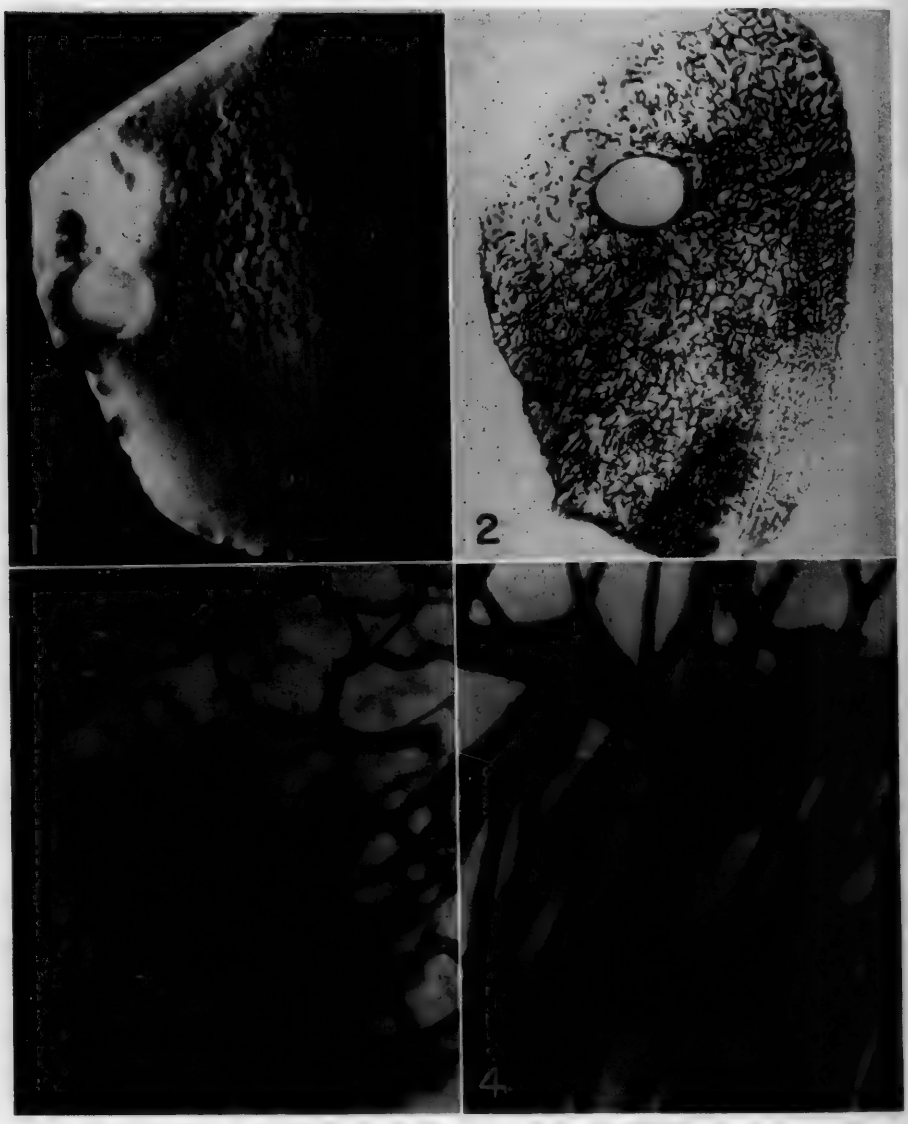

Fig. 1.—Anorganic bone. Bovine cortical bone was cut in sections and treated with ethylenediamine to remove all organic material.

Fig. 2.—An adjacent section of the bone, shown in Fig. 1, impregnated with plastic bearing a particulate dyestuff, then decalcified and reimpregnated with clear plastic. During the initial impregnation, the walls of the haversian canals acted as a filter to hold back the dye particles while the clear plastic penetrated into the smaller spaces previously occupied by organic matter in the specimen. Subsequent demineralization and reimpregnation with clear plastic cleared the specimen. The numerous vascular channels and their branches can be seen.

Fig. 3.—A higher magnification of Fig. 2 reveals the distribution and configuration of some of the vascular channels outlined with dye in the otherwise cleared specimens. The canals are viewed in a plane perpendicular to the long axis of the bone.

Fig. 4.—Another block of cortical bone treated as before but seen here in the long axis of the bone. This specimen is 300 $\mu\mu$ thick and clearly reveals the superficial as well as the deeper channels in the cleared plastic specimen. The smallest channels measure 25 $\mu\mu$ in diameter.

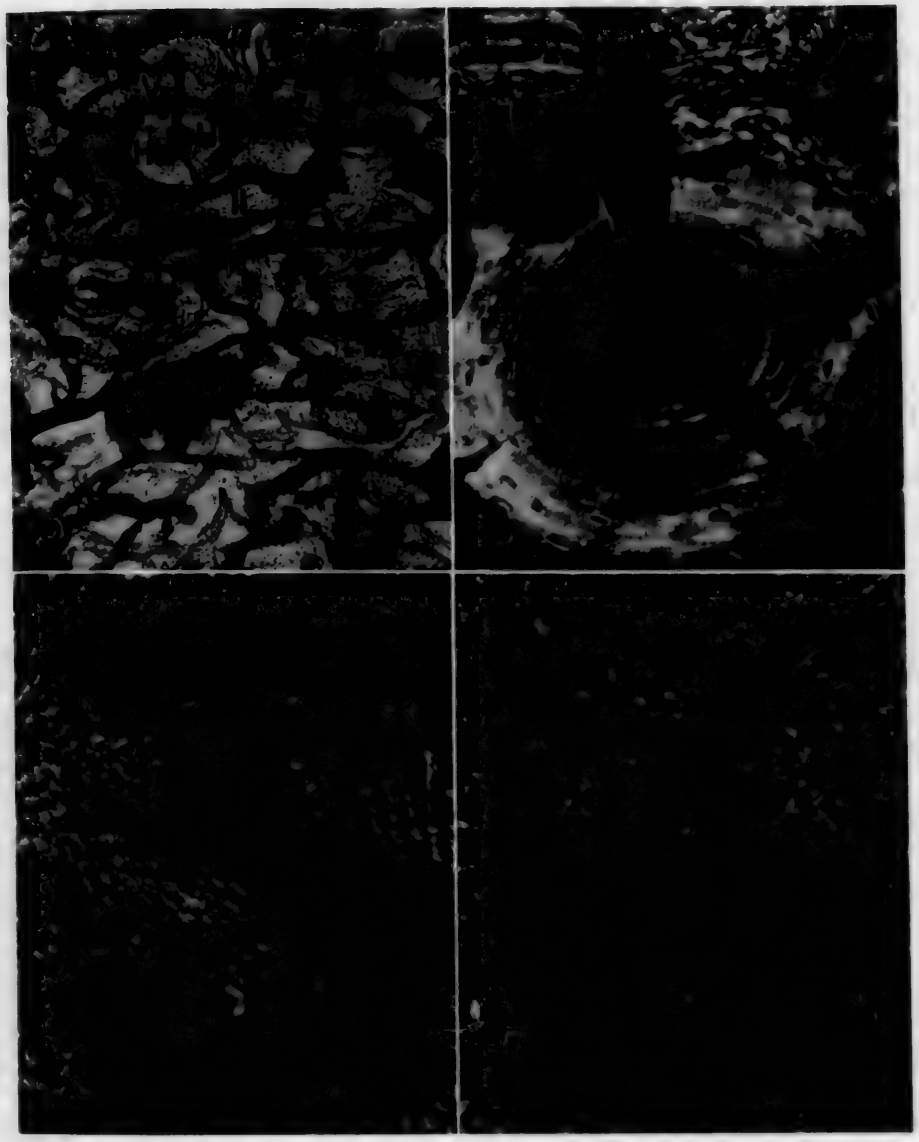

Fig. 5.—After partial secondary plastic impregnation and using oblique illumination, the microscopic structure of haversian systems can be observed as well as the related vascular channels. Volkmann's canals (*V*), haversian canals (*H*), interstitial lamellae (*IL*), and haversian lamellae (*HL*).

Fig. 6.—A higher magnification of Fig. 4 reveals detailed structure of an haversian system. Note the haversian lamellae, lacunae, and canaliculi and two Volkmann's canals joining the haversian canal. Lacunae (*L*).

Fig. 7.—This is a phase contrast photomicrograph of a plastic and gelatin model of mature enamel. The enamel was prepared by ED extraction, impregnation with acrylic plastic, decalcification and infiltration with gelatin.

Fig. 8.—The structure of the enamel rods and interprismatic substance can be observed in this thin section of plastic and gelatin embedded enamel. The embedding material has apparently replaced the organic and inorganic substances of enamel accurately. Observe the longitudinal and cross-sectioned enamel rods (*R*) and the interprismatic substance (*I*).

The canals can be observed more clearly. In this plane, perpendicular to the long axis of the bone, the canals appear to form a regular network throughout the bone model. When a sample of cortical bone is sectioned along its longitudinal axis, rows of parallel haversian canals appear and numerous Volkmann's canals join them at nearly right angles (Fig. 4). Since the inorganic portion of this specimen is transparent, it is possible to visualize the channels deep in the bone, branching and joining the haversian canals of the surface plane. The channels are thus observed to be spaced fairly regularly and are completely interconnected. It was also of interest to study the relationship of the vascular channels to the haversian systems. Thus, one series of anorganic bone samples with vascular channels outlined with dye was decalcified and reimpregnated with plastic to approximately two thirds the depth of the block. This resulted in clearing of the specimen to the level of infiltration of the secondary impregnation. The remainder of the block contained the plastic skeleton of only the initial infiltration. Thus, such microscopic structures as lamellae, lacunae, and canaliculi appeared. The relationship of colored plastic-filled blood vessels to haversian systems is seen in Figs. 5 and 6. Volkmann's canals can be observed communicating with the haversian canals and with canals within interstitial lamellae. A haversian system with its central canal joined by two Volkmann's canals can be observed at high magnification in Fig. 6. This is an enlargement of the central area of Fig. 5. The plastic structure reveals the lacunae and canaliculi of the haversian system.

When it is desirable to study calcified tissues at even higher magnifications, plastic and gelatin-embedded specimens can be sectioned on the freezing microtome, mounted and studied. Five-micra sections of a plastic-gelatin model of enamel can be seen in Figs. 7 and 8. This sample of enamel was from a mature erupted tooth and so consists mainly of inorganic salts which are represented by gelatin. The interprismatic substance between rods is believed to contain proportionately less inorganic salts and thus appears to be represented mainly by plastic which was infiltrated into the enamel following its treatment with ethylenediamine. The rod boundaries and the spaces occupied by interprismatic substance are more distinct than can be observed on thicker calcified ground sections.

SUMMARY

Organic material was extracted from samples of bone and teeth with ethylenediamine. The anorganic bone was impregnated with plastic containing a particulate dyestuff, decalcified, and impregnated with clear plastic to reveal a three-dimensional model of the vascular pattern. By only partial secondary plastic infiltration, the microstructure of bone can be studied in relation to this vascular skeleton. By substituting a low molecular weight plastic and gelatin for the organic, water, and inorganic portions of dentin and enamel, it was found that this model could be sectioned on a microtome and an accurate histologic reproduction obtained. Application of these technics should result in further elucidation of the structure of calcified tissues.

The authors wish to express their sincere appreciation to Miss Elizabeth Haley for sectioning and mounting of the three-dimensional replicas of bones and teeth.

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THE EFFECT OF HYDROCORTISONE PLUS POLYANTIBIOTICS UPON THE DAMAGED AND INFECTED DENTAL PULP OF RAT MOLARS

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SHORTLY after the important role of the adrenal cortical hormones in inflammation was announced, the possibilities of their use in dental practice were considered and attempts were made to use hydrocortisone in the treatment of root canals for the control of apical periodontitis.

Wolfsohn[1] reported reduction of severe inflammatory reactions in the periodontal membrane, but noted increased inflammatory response when he used the hormone on an infected pulp. Blitzer,[2] who used a combination of hydrocortisone and antibacterial agents, pointed out that hydrocortisone aided materially in reducing inflammation of the periapical tissues.

These reports are in agreement with what we know about the action of hydrocortisone, namely, that it reduces inflammation but that it does not inhibit the growth or spread of bacteria. Therefore, success in using the hormone in the treatment of infection would seem more efficacious if it were accompanied by antibiotic therapy.

Because no serious attempt has been made to test the effects of hydrocortisone on the infected dental pulp, a study was undertaken of this hormone alone and in combination with polyantibiotics.

MATERIALS AND METHODS

The experimental material consisted of 96 albino rats, male and female, 60 to 120 days old, which were obtained from the stock of the Medical Center, University of Rochester, Rochester, N. Y. Throughout the course of the experiments, the animals were fed Purina Lab Chow and tap water with no restriction on quantity. For the purpose of the study the animals were divided into 7 treatment groups (Table I).

GROUP A (16 animals).—The lacerated pulps of 23 maxillary first molars were covered with hydrocortisone (H) alone, either as hydrocortisone acetate dental ointment 2.5† per cent or as hydrocortisone powder 2 per cent, pulverized from a tablet, containing 20 mg. hydrocortisone mixed with zinc oxide to 1 gram.

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†Hydrocortone, Merck & Co., Inc.

TABLE I

DATA ON 96 WHITE RATS IN WHICH THE EXPERIMENTAL PULPS OF THE MAXILLARY FIRST MOLARS WERE COVERED WITH HYDROCORTISONE AND POLYANTIBIOTICS

EXPERIMENTAL GROUP	DRUG IN CONTACT WITH LACERATED PULP	POSTOPERATIVE DAY OF SACRIFICE				TOTAL NUMBER OF ANIMALS
		7 DAYS	14 DAYS	28 DAYS	2 MONTHS	
A	Hydrocortisone (H)	4	4	4	4	16
B	H + Neomycin + bacitracin (HNB)	3	3	3	2	11
C	HNB + calcium compounds	3	3	4	4	14
D	H + oxytetracycline + chloramphenicol (HOC)	3	3	3	3	12
E	HOC + calcium compounds	4	3	4	3	14
F	HOC + zinc oxide-eugenol	4	3	3	3	13
G	Oxytetracycline + chloramphenicol + calcium carbonate or zinc oxide-eugenol	4	4	4	4	16
	Totals	25	23	25	23	96

GROUP B (11 animals).—The lacerated pulps of 11 maxillary first molars were covered with hydrocortisone—Neomycin—bacitracin ointment (HNB),* each gram containing 25 mg. hydrocortisone, 3.5 mg. Neomycin base, and 1,000 units zinc bacitracin.

GROUP C (14 animals).—The lacerated pulps of 16 maxillary first molars were covered with HNB paste mixed either with calcium hydroxide or calcium carbonate.

GROUP D (12 animals).—The lacerated pulps of 16 maxillary first molars were covered with a mixture of hydrocortisone, oxytetracycline (Terramycin) and chloramphenicol (HOC), either in the form of ointment or powder, each gram of which contained 20 mg. hydrocortisone, 50 mg. oxytetracycline, and 20 mg. chloramphenicol.

GROUP E (14 animals).—The lacerated pulps of 22 maxillary first molars were covered with the same preparation used on the animals of Group D except that the drugs were mixed either with calcium hydroxide or calcium carbonate.

GROUP F (13 animals).—The lacerated pulps of 17 maxillary first molars were treated with the same preparation as in Group D except that the drugs were mixed with zinc oxide—eugenol.

GROUP G (16 animals).—The lacerated pulps of 20 maxillary first molars were treated with the antibiotics, oxytetracycline and chloramphenicol (OC), mixed either with calcium carbonate or zinc oxide—eugenol.

The treatments were performed on the maxillary first molars. One hundred and twenty-five teeth served as the experimentals while 67 teeth served as controls. Controls included (a) 54 pulps which were untreated, (b) 5 pulps which were exposed but untreated, and (c) 8 pulps which were exposed and covered with oxyphosphate of zinc cement.

Before any operations were performed on the teeth, all animals were anesthetized by intraperitoneal injection of Nembutal and their heads immobilized on a specially designed appliance. Either the anterior wall or the posterior wall of the mesial fossa of the molar was chosen as the point of entry

*Hydroderm, Sharp & Dohme, Division of Merck & Co., Inc.

to the pulp. The pulp was entered by means of a few rotations of a bur attached to a table engine and the exposure was made by hand rotation of specially prepared burs as described by Maurice and Schour.[3]

No attempt to use asepsis or antisepsis was made. The drugs were applied to the exposure either immediately or 1 to 48 hours thereafter. Pressure was used in making the applications so that bleeding could be reduced and the formation of a large blood clot prevented. The cavities were sealed with oxyphosphate of zinc cement or amalgam.

Animals were sacrificed with ether at 7, 14, 28 days, and after 2 months. The heads were removed, the jaws dissected and placed in 10 per cent formalin for fixation. Following decalcification and dehydration, the specimens were embedded in paraffin and serially sectioned at 6 microns in a frontal plane parallel to the long axis of the tooth. They were stained in hematoxylin—eosin and Heidenhain's azan stain.

Criteria of Analysis.—

The following criteria were used to analyze the effects of hydrocortisone and antibiotics on the lacerated dental pulp (Table II).

Complete necrosis: Microscopic evidence showed that the whole pulp had undergone necrosis extending into the apical area.

Progressive degeneration: Healing did not take place, although many pulps attempted to seal off the wound by the formation of a bridge. Breaking down of cellular elements progressed and the pulp might succumb to infection and pass into necrosis.

Incomplete healing: The calcific bridges were still in the primary stage of formation, showing irregular fibrous calcification without regular dentin formation but the final result was healing.

Complete pulp healing: Bridges showing a layer of regular dentin with new odontoblasts and healthy pulp tissue.

FINDINGS

Microscopic examination showed that the exposures in these series of investigations involved laceration and loss of large parts of pulp tissue, often leaving large spaces in which debris accumulated and infection persisted or recurred following early loss of the filling. Obviously these injuries were more extreme than exposures and are called lacerations hereafter.

Two conditions interfered with observations. (1) Dentin chips were often found forced into the pulp by the burring procedure. This was the result of a fracture caused by the bur and was unavoidable in working in these small teeth.[4] (2) The closeness of the pulpal roof and floor as a result of variation in the anatomy of the pulp. When the bur macerated the middle part of the pulp chamber, the two narrow areas situated laterally to the laceration were rapidly calcified by dentin bridges.

The high percentages of pulpal necrosis and progressive degeneration observed after a 4-week postoperative period may, in some instances, have been due to re-infection following early loss of the filling or the fracture of the tooth (see Table II).

TABLE II
HISTOLOGIC FINDINGS UNDER HYDROCORTISONE AND ANTIBIOTICS

DRUG IN CONTACT WITH LACERATED PULP	NUMBER OF TEETH	COMPLETE NECROSIS (%)	PROGRESSIVE DEGENERATION (%)	PULP HEALING	
				INCOMPLETE (%)	COMPLETE (%)
1-Week Postoperative Period					
A. Hydrocortisone (H)	6	0	33	33	33
B. H + Neomycin + bacitracin (HNB)	3	0	0	100	0
C. HNB + calcium compounds	3	33	0	67	0
D. H + oxytetracycline + chloramphenicol (HOC)	4	0	0	25	75
E. HOC + calcium compounds	4	0	0	25	75
F. HOC + zinc oxide-eugenol	4	0	0	50	50
G. OC + calcium carbonate or zinc oxide-eugenol	5	0	0	80	20
	29	3	7	52	38
2-Week Postoperative Period					
A. Hydrocortisone (H)	6	33	17	17	33
B. H + Neomycin + bacitracin (HNB)	3	0	0	67	33
C. HNB + calcium compounds	3	0	0	67	33
D. H + oxytetracycline + chloramphenicol (HOC)	4	0	0	25	75
E. HOC + calcium compounds	6	0	0	17	83
F. HOC + zinc oxide-eugenol	6	0	0	50	50
G. OC + calcium carbonate or zinc oxide-eugenol	5	0	0	60	40
	33	6	3	39	52
4-Week to 2-Month Period					
A. Hydrocortisone (H)	11	27	27	36	10
B. H + Neomycin + bacitracin (HNB)	5	40	20	20	20
C. HNB + calcium compounds	10	40	30	10	20
D. H + oxytetracycline + chloramphenicol (HOC)	8	12	0	38	50
E. HOC + calcium compounds	12	8	8	33	50
F. HOC + zinc oxide-eugenol	7	14	29	14	43
G. OC + calcium carbonate or zinc oxide-eugenol	10	20	10	30	40
	63	22	17	27	33
All 7 Materials	125	14	11	36	39

Normal Control Pulps.—

The molar pulps of the control rats showed a great deal of variation in appearance and had similarities to the human pulp. The blood vessels were large and hyperemic. Capillaries were scattered in the odontoblastic layer, sometimes reaching the predentin. The connective tissue stroma contained the cellular elements usually found in other connective tissue, such as fibroblasts, spindle-shaped mesenchymal cells near the endothelial cells, some histiocytes at some distance from the vessel wall, a few plasma cells and, often, extravasated erythrocytes. Many pulps showed unusual arrangements of the odontoblasts with empty spaces between them. This was observed in both control and experimental pulps and is thought to be a result of the histologic preparation.

Pulps Lacerated but Untreated.—

All the lacerated but unmedicated pulps showed complete loss of nuclear staining throughout the pulp, fibrosis, progressive degeneration, abscess formation and, in some instances, periapical involvement because of liquefaction necrosis.

Pulps Lacerated and Covered With Oxyphosphate of Zinc Cement.—

Sections of tissues taken at seven days showed that the superficial pulp tissue in contact with the cement was necrotic. When the pulp was only slightly damaged, the necrotic area was demarcated from the rest of the pulp by coarse fibril bundles and, at 14 days' condensation of the tissue around these fibrils, was forming osteoid-like structure. Under this a tubular form of dentin with odontoblastic processes was seen after 4 weeks.

When damage to the pulp was severe and infection was provoked due to loss of the filling, extensive pulpal degeneration was already seen after 7 days, which rapidly advanced and, at 28 days, the pulp succumbed to the infection. Healing took place in 3 out of 8 cases treated with oxyphosphate of zinc cement.

Pulps Lacerated and Treated With Hydrocortisone Acetate Ointment or Powder (Group A).—

The findings from cases treated with either the ointment (2.5 per cent) or the powder (2 per cent) do not differ and will be described together.

Seven-day postoperative period: When the pulps were not exposed to severe damage and infection, there was no marked difference between the experimental pulps of this group and the lacerated pulps covered only with oxyphosphate of zinc cement. An initial surface necrosis developed, and there seemed to be no restriction in the defense activity of the mesenchymal tissue. The number of capillaries in the irritation area was markedly increased and they were engorged with red blood cells. This feature was prominent in all groups of experiments where healing took place. It was most prominent in the first days of experimental periods.

In some instances, in spite of the destruction of a considerable amount of tissue by the bur and the forcing of many large dentin spicules into the pulp,

bridges of osteoid-like structure were formed directly beneath the area of destruction and odontoblasts at the side of the bridge were laying down large amounts of secondary dentin (Fig. 1). This was probably due to mild infection. When infection was provoked by allowing a period of 48 hours to elapse between the experimental injury and the introduction of hydrocortisone (2.5 per cent), extensive pulpal degeneration was observed. Although some calcification took place, no dentin bridges were formed (Fig. 2).

In 1 case where a higher concentration of hydrocortisone (8 per cent) was used and a large portion of the pulp was macerated, a thick layer of necrotic pulp tissue could be observed under the area of laceration with disruption of the odontoblastic layer and disintegration and necrosis of the cells. Beneath the necrotic area, a mass of osteoid-like structure formed down to the root (Fig. 3).

Fig. 1.—Photomicrograph showing pulpal reaction to hydrocortisone powder 2 per cent, 1 week after laceration of the pulp. (Hematoxylin-eosin; ×40.) Large parts of broken dentin (*P*) are pushed into the pulp tissue. Note the formation of a primary calcific bridge (*BR*). *D*—dentin.

Fourteen-day postoperative period: The dental bridges were still forming but in 1 case extensive pulpal degeneration was seen below the calcific bridge as probably re-infection resulted after loss of the filling. In another case where the higher concentration of the hormone (8 per cent) was used and infection was provoked by allowing a period of 24 hours to elapse between the experimental injury and the introduction of the hormone, the whole pulp appeared necrotic.

Twenty-eighth day to 2-month postoperative period: The regenerative capacity of the pulp was not affected as long as infection was mild. Dentin bridges had formed and were still growing. The rest of the pulp was normal (Fig. 4). When infection was severe, as when provoked by loss of the filling, dentin bridges were incomplete or the pulp was necrotic. In the latter instances, when the higher dosage of the hormone was used, necrosis appeared increased and infection extended to the apical region.

It may be noted that pulpal healing was dependent upon the degree of infection. When the dosage of the hormone was increased and infection was not severe, the pulp gave evidence of continuing efforts at repair which indicates that hydrocortisone did not markedly interfere with the reparative ability of the tissue cells.

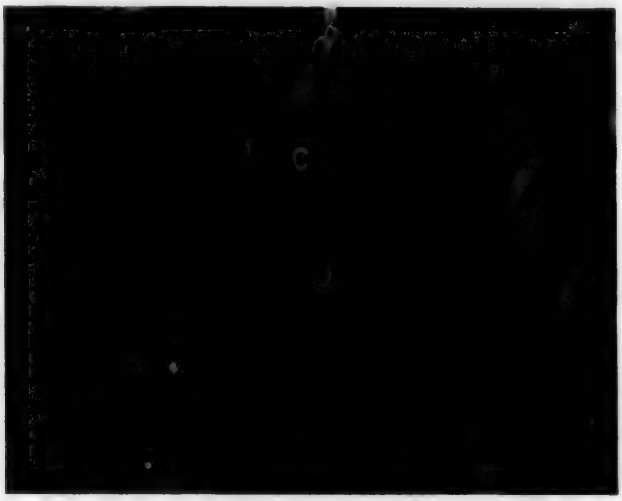

Fig. 2.—Photomicrograph showing pulpal reaction to hydrocortisone acetate ointment 2.5 per cent, 1 week after laceration of the pulp. Forty-eight hours elapsed between the injury and the placement of the drug. (Hematoxylin-eosin; ×40.) Note extensive pulpal degeneration. Note also calcification of degenerated tissue (*C*).

Fig. 3.—Photomicrograph showing pulpal reaction to hydrocortisone powder 8 per cent, 1 week after laceration of the pulp. (Hematoxylin-eosin; ×100.) Note the large mass of necrotic tissue and beneath this area, the formation of osteoid-like structure (*BR*). *D*—dentin; *RB*—extravasated red blood cells.

At 28 days, complete healing was observed in 16 per cent of the teeth, treated either with hydrocortisone ointment or powder. Of all 3 intervals combined, 22 per cent of the teeth showed complete healing (see Table II).

Pulps Lacerated and Treated With a Combination of Hydrocortisone—Neomycin—Bacitracin (HNB) (Group B).—

Seven-day postoperative period: The pulps, although severely injured, still showed normal tissues beneath the necrotic area. Beginning of formation of irregular dentin bridges could be observed, but the process was slow while, under hydrocortisone alone, the bridges had already developed at 7 days. The amount of secondary dentin was, also, much smaller as compared with those cases in the seven-day period of Group A.

Fig. 4.—Photomicrograph showing pulpal reaction to hydrocortisone acetate ointment 2.5 per cent, 4 weeks after laceration of the pulp. (Hematoxylin-eosin; ×100.) Note the narrow dimension between the pulpal roof (*R*) and the pulpal floor (*F*). Note spreading of calcification into pulpal tissue.

Fourteen-day postoperative period: Irregular dentin bridges had developed and there was no marked difference from those teeth treated in Group A and seen after 2 weeks. Large necrotic areas of tissue were observed beyond the bridge when infection was provoked and, in 1 case, masses of extravasated blood were dispersed along the necrotic area. It seemed that the infection was not arrested.

Twenty-eight-day to 2-month postoperative period: Apparently a mild infection did not affect the regenerative capacity of the pulp, and dentin bridges were formed, thus protecting the rest of the pulp which was normal. In cases of provoked infection resulting from loss of the filling, calcified bridges were either incomplete, disturbed, or absent. In 1 case of severe infection, resulting from a 24-hour delay between injury and introduction of material, the pulp had made vigorous attempts to wall off the lacerated part by formation of a

wide mass of calcified tissue, but the pulp beneath this barrier had undergone degenerative changes even although the upper distal horn of the pulp was still normal (Fig. 5).

The antibiotics, Neomycin and bacitracin, in this combination were not sufficiently effective to eliminate the severe infection.

At 28 days, 20 per cent of the teeth treated with HNB paste showed complete healing, and 18 per cent of all 3 intervals combined (see Table II).

Fig. 5.—Photomicrograph showing pulpal reaction to HNB paste, 4 weeks after laceration of the pulp. Twenty-four hours elapsed between the injury and the placement of the drug. (Hematoxylin-eosin; ×100.) The pulp had walled off the laceration by a mass of calcified tissue in form of a bridge (*BR*) but did not succeed and succumbed to the infection. Note the large mass of necrotic pulp beneath the bridge (*NP*). *D*—dentin; *L*—site of laceration.

Pulps Lacerated and Treated With HNB Ointment and Combined With Calcium Compounds (Group C).—

When calcium carbonate was added to the HNB ointment, necrosis was found in almost all pulps which were subjected to severe infection, regardless of the period elapsing before study. Dentin bridges were mostly incomplete or of fibrous structure in the form of a capsule (Fig. 6). In 1 case, where the calcific barrier was complete, it had broken down and the underlying pulp tissue had been destroyed because the barrier had failed to wall off infection. Better results were obtained when calcium hydroxide was added to the HNB paste. A strong dentin bridge was found after 28 days and the pulp was normal. At 28 days, complete healing was found in 20 per cent of the teeth treated with HNB plus calcium compounds and in 19 per cent of all 3 intervals combined (see Table II).

Pulps Lacerated and Treated With a Combination of Hydrocortisone—Oxytetracycline—Chloramphenicol (HOC) (Group D).—

Seven-day postoperative period: The superficial necrosis of the lesion was less pronounced than in Group B. In 2 cases it was absent. Calcification was more advanced and dentin bridges had already developed. A striking feature

was that, contrary to the findings in Groups A, B, and C, the pulp withstood the severe infection resulting from 48 hours' delay between the injury of the pulp and the introduction of the drug. Dentin bridges were already formed in spite of the severe damage to the tissue, and gross infection and finger-like spread of the calcification process into the pulpal tissue could be seen (Fig. 7).

Fig. 6.—Photomicrograph showing pulpal reaction to HNB paste + calcium carbonate, 4 weeks after laceration of the pulp. (Hematoxylin-eosin; ×100.) A capsule of fibrous structure (*CA*) has formed but the pulp succumbed to infection. Note the formation of secondary dentin (*SD*). Note also the necrotic pulp tissue below (*NP*). *D*—dentin; *L*—site of laceration.

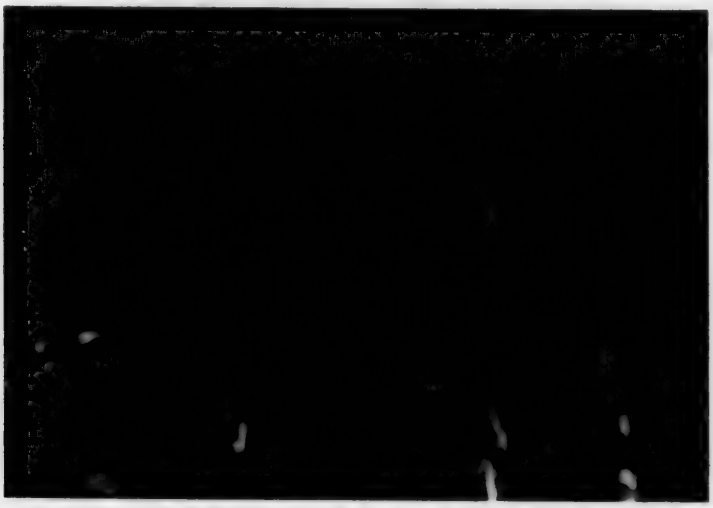

Fig. 7.—Photomicrograph showing pulpal reaction to HOC paste, 1 week after laceration of the pulp. Forty-eight hours elapsed between the injury and the placement of the drug. (Hematoxylin-eosin; ×400.) A bridge has been formed and calcification is spreading finger-like into the pulpal tissue.

Fig. 8.—Photomicrograph showing pulpal reaction to HOC paste, 2 weeks after laceration of the pulp. (Hematoxylin-eosin; ×100.) Note the complete bridging and formation of predentin (*PD*) by new odontoblasts which are adjoining the odontoblasts from the dentin wall. Note also embedded dentin spicule (*SP*).

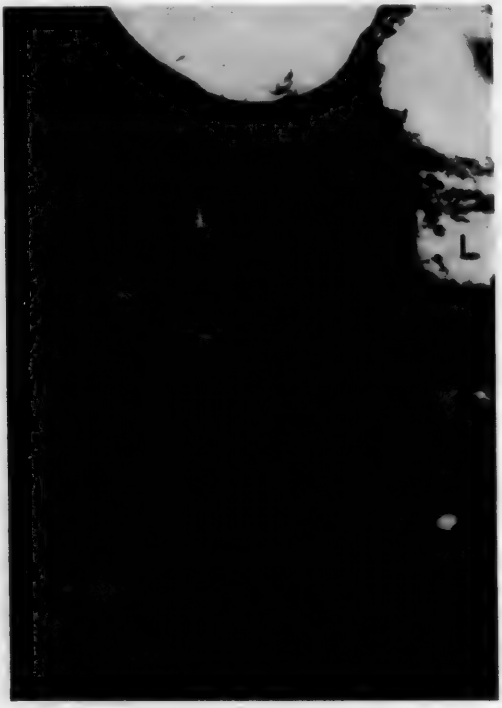

Fig. 9.—Photomicrograph showing pulpal reaction to HOC paste, 4 weeks after laceration of the pulp. (Hematoxylin-eosin; ×100.) The whole coronal pulp is calcified (*C*), only a small part remains vital (*P*). *D*—dentin; *L*—site of laceration.

Fourteen-day postoperative period: The operative channel was free of necrotic material and in direct communication with the calcified tissue. The irregular dentin bridge which had first formed was re-inforced not only by tubular dentin laid down by new odontoblasts but also by deposition of secondary dentin from the old odontoblasts along the dentinal wall (Fig. 8). Some dentin chips which were forced into the pulp tissue could still be seen embedded in the calcified tissue.

Twenty-eight-day to 2-month postoperative period: The calcified tissue occupied almost the whole coronal pulp to a point beyond which a bridge of dentin was formed (Fig. 9).

At 28 days, complete pulp healing was observed in 50 per cent of the teeth treated with HOC paste and in 63 per cent of all 3 intervals combined (see Table II).

Fig. 10.—Photomicrograph showing pulpal reaction to HOC paste + calcium carbonate, 4 weeks after laceration of the pulp. (Hematoxylin-eosin; ×40.) In spite of severe damage, two bridges (*BR*) on both sides of laceration (*L*) have formed. *P*—normal pulp.

Pulps Lacerated and Treated With HOC Ointment Combined With Calcium Compounds (Group E).—

There was not much difference between the action of HOC plus calcium compounds upon the pulpal tissue and that of HOC paste alone. With calcium hydroxide, dentin bridge formation was complete after 7 days. With calcium carbonate, the dentin bridge was still incomplete after 14 days. After 28 days, bridges of irregular dentin had developed laterally to the injury with healthy pulps underneath (Fig. 10). Except for the thickness of the irregular dentin layer, HOC with calcium carbonate gave essentially the same results as HOC with calcium hydroxide. Of 22 cases treated, 64 per cent showed complete healing.

Pulps Lacerated and Treated With HOC Ointment Combined With Zinc Oxide-Eugenol (Group F).—

The incorporation of zinc oxide-eugenol into the preparation of HOC seemed to modify favorably the degree and type of reaction of the pulp. There was less localized necrosis than when calcium compounds were used, although occasionally severe laceration of the pulpal tissue was seen. Calcification was noted as early as 1 week postoperatively. It is of interest that a calcific bridge was found under zinc oxide-eugenol as well as under calcium carbonate and calcium hydroxide. In spite of provoked infection resulting either from 48-hour delay or loss of the filling, regeneration of the pulp occurred. After 4

Fig. 11.—Photomicrograph showing pulpal reaction to HOC paste + zinc oxide-eugenol, 4 weeks after laceration of the pulp. One hour elapsed between the injury and the placement of the drug. (Hematoxylin-eosin; ×40.) Nearly the whole coronal pulp is calcified (*C*), leaving the remaining pulp healthy (*P*). *D*—dentin; *L*—site of laceration.

weeks, almost the whole coronal pulp was calcified leaving the remaining pulp healthy (Fig. 11). Of 17 cases treated, 47 per cent showed complete healing (see Table II).

Pulps Lacerated and Treated With Antibiotics Without Hydrocortisone (Group G).—

The pulps treated with oxytetracycline and chloramphenicol mixed with calcium carbonate or zinc oxide-eugenol showed, in 3 cases, slight infiltration of polymorphonuclear leukocytes in the first stage of experiment which in the later stages was no longer evident. Surface necrosis was not observed under a small exposure and even in more severe laceration of the pulp the superficial zone was very slightly necrotized. In 1 case, a fibrous borderline was seen between the injury and the bridge formation but, in others, a dentinal bridge formed directly below the wound. Secondary dentin was seen continuous

with the primitive dentin of the bridge (Fig. 12). The spicules of dentin which had been forced into the pulp in making the exposure were often cemented together with dentin or osteoid-like structure (Figs. 13 and 14). The basic pattern of pulpal healing was the same as described in Groups D, E, and F. The only difference was that healing appeared to be delayed in the first days of experiment.

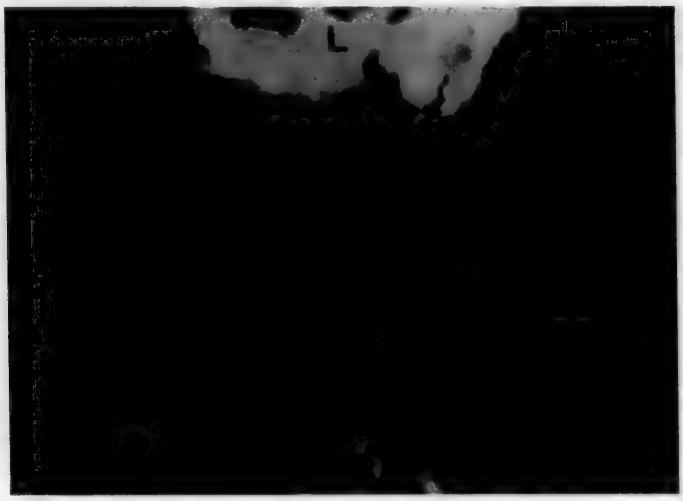

Fig. 12.—Photomicrograph showing pulpal reaction to OC + calcium carbonate, 2 weeks after laceration of the pulp. (Hematoxylin-eosin; ×100.) Note the strong bridge formation (*BR*) which is cemented together with new formation of secondary dentin (*SD*). *P*—normal pulp; *D*—dentin; *L*—site of laceration.

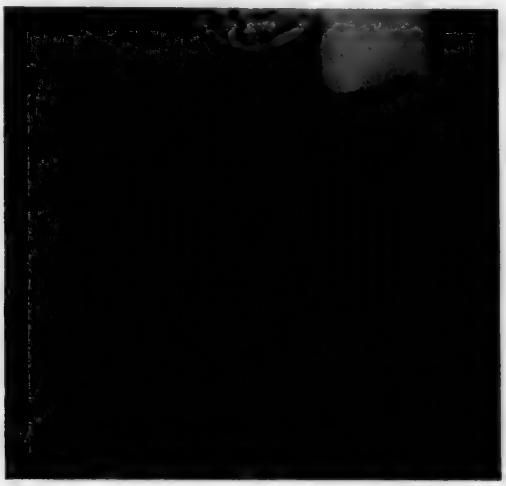

Fig. 13.—Photomicrograph showing pulpal reaction to OC + zinc oxide-eugenol, 2 weeks after laceration of the pulp. (Hematoxylin-eosin; ×40.) A large spicule of dentin (*circle*) is encapsulated by bridge formation which is cemented together with new formation of secondary dentin. *D*—dentin; *L*—site of laceration.

When damage was severe, osteodentin was seen apically in the root. It was difficult to distinguish between teeth treated with calcium carbonate or zinc oxide-eugenol in conjunction with the antibiotics.

With oxytetracycline and chloramphenicol, 35 per cent of the 20 teeth treated showed complete healing (see Table II).

Fig. 14.—Photomicrograph showing a high-power view (hematoxylin-eosin; ×400) of bridge area seen circled in Fig. 13. *SP*—spicule of dentin; *BR*—bridge formation; *SD*—secondary dentin.

DISCUSSION

This study has shown that a combination of hydrocortisone and antibiotics increased the survival of pulps after injury and infection. However, the part played by hydrocortisone in producing these results is uncertain, particularly because necrosis was far more common with the steroid when pulp injury and infection were severe. On the other hand, our experiments presented no evidence that hydrocortisone of itself actually interfered with tissue repair. This conclusion is of some interest because studies of wound healing have shown that the 3 essential components of connective tissue—cells, fibers, and ground substance—are susceptible to the action of the hormone. Similar effects might have been expected in the pulp as an embryonic type of connective tissue.

In our experiments, no restriction of the formation of new capillaries as seen in wound healing could be observed, and cell differentiation, as indicated by bridge formation and formation of secondary dentin, was in no way inhibited by the hormone. This suggests that further studies of the effects of hydrocortisone on pulpal and other mesenchymal tissues might be profitable and could cast some light on the as yet uncertain mechanism of its action and specific influence on mesenchymal tissue reaction.

It may be worth noting that, in our experiments, pulp survival was better when oxytetracycline and chloramphenicol were used than when pulps were

treated with Neomycin and bacitracin. We did not find that the incorporation of zinc oxide-eugenol inactivated the antibiotics, oxytetracycline and chloramphenicol, as reported by Shaw, Sprawson, and May.[5] A comparison of the results obtained after calcium compounds or zinc oxide-eugenol were added to hydrocortisone and antibiotics, indicated that they did not interfere with the process of healing. It was noted that bridging with calcium hydroxide was stronger than with calcium carbonate and zinc oxide-eugenol.

The results of this preliminary investigation concerning the effect of hydrocortisone on the dental pulp of rat molars are inconclusive and do not constitute an evaluation of the role of steroid medication in pulp procedures. Further studies of the effect of hydrocortisone on the exposed human dental pulp are in progress.

SUMMARY AND CONCLUSION

The effect of hydrocortisone, alone and in combination with polyantibiotics upon experimentally damaged and infected dental pulps was studied histologically in the first upper molars of 96 white rats. A large portion of pulp was macerated and exposed to infection. To avoid bleeding and to prevent the formation of a blood clot, the drugs were purposely forced into the lacerated tissue.

Treatment of lacerated pulps with hydrocortisone alone showed 22 per cent complete healing. When the hormone was combined with polyantibiotics, the lacerated pulps covered with the hormone and a mixture of oxytetracycline and chloramphenicol showed a significantly higher incidence of complete pulp healing (63 per cent) than those with Neomycin and bacitracin (18 per cent). Lacerated pulps covered with oxytetracycline and chloramphenicol without hydrocortisone gave 35 per cent complete healing.

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CHLORIDE LEVELS IN PAROTID SECRETION

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INTRODUCTION

SALIVA has been used as a medium for the presumptive diagnosis of various diseases and physiologic states but, in most instances, whole saliva has been used. The ease with which parotid secretion can be collected has uncovered an intriguing area for study wherein the chemistry of a "pure secretion" can be studied in relation to systemic and oral disease.

In a recent study, Anders[1] found that the mean chloride level in the whole unstimulated salivas of persons with rapid carious breakdown was significantly higher than in a caries-free group.

The present study was designed primarily to compare chloride levels of parotid secretions obtained by controlled conditions of mechanical stimulation from two groups of individuals with extreme differences in dental caries experience to determine whether caries activity is related to the chloride level of a "pure" salivary secretion.

METHODS

Thirty-one caries-free and 50 caries-rampant male recruits from the U. S. Naval Training Center, Great Lakes, Ill., were utilized for this study. The caries-free individuals had no carious lesions that could be detected by clinical and radiographic means and had zero lactobacilli counts and normal gingivae. An additional 10 individuals with questionable or few incipient interproximal enamel lesions were added to the caries-free group. The resulting group of 41 was considered one relatively free from caries experience. The average age of the experimental groups was 18 years. The subjects in the caries-rampant group possessed 10 or more teeth with extensive caries and most had molar teeth in which the decay had completely hollowed out the crowns. The majority exhibited decay of the lower anterior teeth. It was, therefore, possible to compare two extreme groups.

Each sample of parotid saliva was obtained from the right Stensen's duct by means of a parotid cup[2] and was collected in a graduated centrifuge tube. The

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parotid gland was stimulated for 5 minutes by having each subject chew 2 sticks (3.0 Gm.) of flavored chicle.* All collections were made from resting individuals between 8:00 and 8:30 A.M.

Parotid saliva was also collected 50 times on each of 2 individuals using different methods of stimulation to produce different flow rates of parotid saliva during a 5-minute period.

The volume of each saliva sample was recorded, and duplicate chloride determinations were made on 2 ml. aliquots that were acidified with 1 ml. of concentrated HNO_3. All samples were titrated potentiometrically with 0.01 N $AgNO_3$ to an endpoint of 0.71 volt on a Fisher titrimeter using silver—silver chloride electrodes. This endpoint was determined from standard titration curves made from pooled parotid saliva. No attempts were made to deproteinize or digest the samples. This method was compared to that of Wilson and Ball[3] in which the proteins were oxidized and wet digested by concentrated HNO_3 in the presence of excess $AgNO_3$, and to potentiometric determinations in which the proteins were digested for 30 minutes. The chloride values showed little variation among these methods; thus, it appeared that the protein concentrations did not influence the accuracy of the potentiometric quantitations. The protein concentration of parotid secretion ranges from 66 to 374 mg. per cent.[4]

RESULTS

The mean parotid chloride concentrations (Table I) for the group relatively free from caries was 26.1 mEq./L. and for the caries-rampant group was 36.1 mEq./L. Statistical analysis of group means revealed that this difference was highly significant at less than 1 per cent. Regardless of this statistical difference in group means, the frequency of individual overlapping between the two groups obviated a consideration of parotid chloride concentrations as an indication of dental caries proneness.

TABLE I

MEAN TITRATABLE CHLORIDE LEVELS IN THE PAROTID SALIVAS OF TWO EXTREME GROUPS

	RELATIVELY CARIES-FREE (41 INDIVIDUALS)	CARIES-RAMPANT (50 INDIVIDUALS)
Chloride concentration	26.1 mEq./L. (S.D. ± 11.5)	36.1 mEq./L. (S.D. ± 14.5)
Rate of flow	4.6 ml./5 min.	4.7 ml./5 min.

Difference in chloride group means significant at < 0.01.

The average flow rates for the two groups for the 5-minute periods are also shown in Table I. These rates correspond to those of a previous study[5] and emphasize the constancy of mean flow rates in parotid samples stimulated by the method described.

In the 2 individuals in whom the effect of flow rate on parotid chloride concentration was studied, a statistical analysis of spot graphs with a summary

*Dentyne gum supplied by The American Chicle Company.

of the swarm points by a fourfold table showed a positive correlation between chloride concentrations and flow rate and confirms previous studies.[6, 7]

DISCUSSION

The chloride concentration of parotid saliva is proportional to flow rate and varies between 10 to 43 mEq./L., but is lower than the plasma chloride level of approximately 100 mEq./L.[6] The concentration in whole saliva ranges from 9.0 to 17.0 mEq./L.

It has been reported by Turner and Crane[8] that there is a relationship between the amount of dental caries and the rate at which whole saliva hydrolyzes starch. A high amylase activity would favor the breakdown of starch to free maltose which could be fermented readily by the oral microbiota, and this could be a contributing factor in creating a more propitious environment for the development of dental caries in certain individuals. Chloride ion appears to be necessary for the activation of alpha-amylase in saliva.[9] However, Bernfeld, Staub, and Fischer[10] have shown that a NaCl concentration of 10 mEq./L. will induce 100 per cent activity of alpha-amylase. Since the chloride concentration of the relatively caries-free group in this study was above the range of maximum activity, no increase in amylase activity would be expected in the caries-rampant group and this could not be the explanation for the increased decay.

This study in a pure salivary secretion agrees with the findings of Anders[1] in whole saliva, and does not support the work of Hubbell[11] who found no correlation between the chloride level of whole saliva and caries experience.

SUMMARY

The chloride levels in the parotid salivas of 41 relatively caries-free and 50 caries-rampant males were compared. The mean chloride concentration was significantly greater in the caries-rampant group, but the high frequency of individual overlapping between the two groups ruled out the possibility of using this as a test for caries activity. For a given individual, chloride ion concentration showed a positive correlation with parotid saliva flow rate. The mean parotid flow rates for 5-minute periods of stimulation were the same in individuals with extreme differences in dental caries experience.

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DENTAL CARIES ACTIVITY, AND THE pH, TITRATABLE ALKALINITY, AND RATE OF FLOW OF HUMAN PAROTID SALIVA

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INTRODUCTION

IN various studies undertaken to determine the relationship of the pH, titratable alkalinity, and flow rate of whole saliva to dental caries activity, many investigators[1-6] have reported little or no relationship between the pH of whole saliva and the degree of dental caries activity. In contrast, a definite correlation between the buffer capacity (titratable alkalinity) of "resting" and stimulated whole saliva, and dental caries activity has been noted.[7-12] In addition, certain workers have observed higher salivary flow rates in persons exhibiting a resistance to dental caries.[13-15]

The development of the parotid cup[16-18] has facilitated the collection of the pure secretion (serous) from the parotid glands and made possible the accurate measurement of the secretion rate. This, in turn, has made it possible to relate the secretion rate with certain physiologic and pathologic conditions. The parotid secretion has been considered to be responsible for approximately 34 per cent of the sum of the minute volumes of the submaxillary, sublingual, and parotid secretions under conditions of chemical stimulation of oral receptors,[19] and amounts up to 60 per cent of the whole saliva have been reported.[20] Therefore, it must be considered a substantial contributor to the admixture called whole saliva.

This investigation was undertaken primarily to determine possible relationships between caries activity, and the pH, titratable alkalinity, and the flow rate of the parotid secretion.

METHODS AND MATERIALS

Thirty caries-free and 52 caries-rampant male recruits from the U. S. Naval Training Center, Great Lakes, Ill., were utilized for this study. The average age of the experimental groups was 18 years. Caries-free persons were selected by

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clinical and radiologic examinations and they had no restorations, normal gingivae, and zero lactobacilli counts. The subjects contained in the caries-rampant group possessed 10 or more teeth with extensive caries and most had molar teeth in which the decay had completely hollowed out the crowns. The majority exhibited decay of the lower anterior teeth and all of them had high lactobacilli counts.

Saliva samples were collected between 8:00 and 8:30 A.M. Most of the subjects had to be awakened from sleep and all remained in bed, in a sitting position, during the period of collection. None had eaten for at least 12 hours.

Saliva was obtained by means of a parotid cup, and samples were collected under 1 ml. of mineral oil in a graduated centrifuge tube chilled in ice. Experiments were run to determine whether oil and ice would retard the escape of CO_2 with a subsequent rise in pH. It was found that within an hour after reaching room temperature, the pH of an oil-protected sample was elevated to a maximum of 0.1 pH unit. Saliva samples allowed to stand in air for long periods of time showed no change in titratable alkalinity. The 5-minute secretion rates from the right and left parotid glands in various individuals were compared. Although each person always produced a slightly greater secretion in one gland, the differences were never greater than a few tenths of a milliliter. It was found that in a random sample of the population, the mean rates of secretion from the right and left glands stimulated similarly with flavored chicle could be considered identical. Accordingly, the secretion from only one gland was utilized for the present study, and stimulation was accomplished by having the subject chew 2 sticks (3.0 Gm.) of flavored chicle* for 5 minutes on the side of collection. This type of stimulus elicited a representative gustatory, olfactory, and mechanical response.

A "get-acquainted" collection was made on the day prior to the actual collection. This psychologically conditioned the subject and minimized anxiety.

The volume of each 5-minute saliva sample was recorded and, within 15 minutes after the oil-protected sample had reached room temperature, the pH of a 0.5 ml. aliquot was measured on a Beckman Model G potentiometer. Titratable alkalinity was determined electrometrically with a Fisher titrimeter. Duplicate 1 ml. samples were titrated with 0.01 N HCl to an endpoint of pH 4.8 (0.33 volts). This endpoint was determined from a standard titration curve based on the pooled parotid salivas from 20 persons.

RESULTS

The pH and titratable alkalinity were determined 30 times on each of 5 subjects, using varying degrees of stimulation to produce different rates of flow of parotid salvia. The pH and buffer values were plotted against the rate of flow for each person. Statistical analysis of spot graphs showed a definite correlation between pH and titratable alkalinity, and rate of flow for each individual. The average coefficient of correlation between rate of flow and titratable

*Dentyne gum supplied by the American Chicle Co.

alkalinity for the 5 individuals was 0.92. The slope of the best straight line for each of the 5 sets of data was calculated by the method of least squares. The values for the slopes of the 5 lines were 1.42, 1.50, 2.09, 2.35, and 2.71 and are statistically different. Fig. 1 shows the relationship between rate of flow and titratable alkalinity for 2 of the 5 subjects. Accordingly, it appears that although there is a general increase in titratable alkalinity with the flow rate of parotid saliva, the titratable alkalinity of each individual varies at a rate characteristic for that individual.

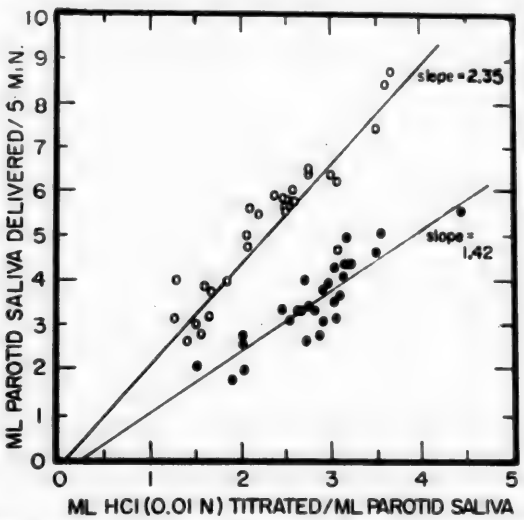

Fig. 1.—The relation between flow rate and titratable alkalinity of parotid saliva of 2 individuals.

It was found that, in the 15 persons tested, variations within a 40 to 90 strokes-per-minute range did not cause fluctuation in the secretion rate. In many instances, increases of 20 strokes per minute caused a barely perceptible decrease in the flow rate. The average mastication rate of the 15 persons tested was 70 strokes per minute.

No significant differences in flow rate, pH, and titratable alkalinity were noted between the caries-free and caries-active groups. The means and standard deviations for the three variables are presented in Table I.

TABLE I

THE MEAN RATE OF FLOW, pH, AND TITRATABLE ALKALINITY OF THE PAROTID SALIVAS OF CARIES-FREE AND CARIES-RAMPANT PERSONS

	CARIES-FREE (30 PERSONS)	CARIES-RAMPANT (52 PERSONS)
Rate of flow (ml./5 min.)	4.64 S.D. ± 1.86	5.11 S.D. ± 2.24
pH	7.33 S.D. ± 0.245	7.37 S.D. ± 0.176
Titratable alkalinity (ml. 0.01 N HCl/ml. parotid)	2.38 S.D. ± 0.718	2.40 S.D. ± 0.818

DISCUSSION

Since previous reports have indicated a relationship between dental caries and the flow rate of whole saliva, and a stronger correlation between dental caries activity and the titratable alkalinity of whole salvia, one might suspect that such parallel relationships would exist with parotid secretion. However, the flow rate and titratable alkalinity of parotid saliva for caries-free and caries-rampant persons did not differ significantly under the test conditions imposed in the present study. It should be noted that most of the caries-free individuals selected for this study were from areas in which the water contained an optimum amount of fluoride. The teeth of these individuals would be intrinsically more resistant to decay, and this group would not need the more favorable salivary environment to be caries resistant.

Schneyer and Levin[19] have shown that the secretion from the submaxillary and sublingual glands contributes the bulk (approximately 65 per cent) of the saliva secreted by the three pairs of the major salivary glands (fraction total). Although they first felt that approximately 40 per cent of the total mixed saliva may come from the small mucosal glands, Schneyer later demonstrated that the secretions derived from the three major salivary gland pairs fully accounted for the volume flow of the resting total mixed saliva.[21] In all probability, the mucosal glands contribute only negligibly to the volume of exogenously stimulated whole saliva. One would, therefore, expect the submaxillo-sublingual saliva to have the greatest influence on the volume and buffer capacity of whole saliva. Perhaps the rate of flow and titratable alkalinity of submaxillo-sublingual saliva is more closely associated with caries activity and could account for the findings of previous investigators. This thesis should be tested since the lower incisors, which are bathed directly by submaxillo-sublingual saliva, are susceptible to carious attack for the longest period of time yet manifest the lowest incidence of caries.

The bilateral constancy of the secretion rate confirms similar previously reported results.[17, 22] This investigation supported Kerr's observation[23] that chewing rate has little influence on the parotid saliva secretion as long as the number of strokes per minute fall within the normal physiologic range of 40 to 90 strokes. Furthermore, it was shown that each person possesses a narrow range of masticatory rate.

The observation of a relationship between the pH and titratable alkalinity and the rate of flow is in agreement with other investigations. Dreizen, Reed, Niedermeier, and Spies[24] have shown that the buffer capacity, bicarbonate and sodium concentrations are greater in stimulated than in resting whole saliva, and Hildes and Ferguson[25] have demonstrated that the sodium, bicarbonate and pH of human parotid saliva vary directly with the rate of flow.

In studies of this type, it is necessary that the collection of all saliva samples be made under comparable conditions. The method of stimulation must be standardized in order to detect intrinsic differences in the secretory potential of each individual. Changes in the intensity of the stimuli not only alter the rate of salivary flow but also alter the concentration of the inorganic components

upon which the pH and titratable alkalinity are dependent.[24-26] Other factors which require control are the time of collection[27-28] and, insofar as possible, the metabolic state of the individual.[29]

Chauncey, Lisanti, and Winer[22] have hypothesized that the carbon dioxide and water produced by the normal metabolic processes of the parotid glands are converted into carbonic acid with a subsequent ionization into hydrogen and bicarbonate ions. They further proposed that the hydrogen ions are transferred to the blood or the extravascular fluid while sodium ions are transferred from the blood to the salivary gland cells and are secreted with the bicarbonate in the saliva. This would account for the increased pH, sodium, bicarbonate and titratable alkalinity produced by an increased secretory rate, and is in accord with the findings of Wah Leung[30] who pointed out the importance of bicarbonate as one of the principal buffer components of stimulated saliva.

SUMMARY

The pH, titratable alkalinity, and rate of flow of the parotid salivas from 30 caries-free males and 52 with rampant-caries were determined under standard conditions of collection. No differences in pH, titratable alkalinity, and rate of flow existed between these two extreme groups.

Parotid saliva specimens were collected from each of 5 subjects using varying degrees of stimulation which produced different rates of flow. In all cases, both titratable alkalinity and pH varied directly with rate of flow. Titratable alkalinity increased at a rate characteristic for each person studied.

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PARABIOSIS IN STRAINS OF CARIES-SUSCEPTIBLE AND CARIES-RESISTANT RATS

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THE development of caries-resistant and caries-susceptible strains of rats[1, 2] and hamsters[3] provided direct evidence that heredity is a strong determinant of the degree of susceptibility to caries. Mechanisms have been sought to explain this constitutionally determined difference in susceptibility between strains. Nakfoor, Hunt, and Hoppert[4] observed that the lower molars of their caries-susceptible rats fractured more readily under a standard impact than those of the resistant strain, but concluded that fracture is probably not an important factor in the initiation of caries. Kifer, Hunt, and Hoppert[5] noted, in 40-day-old Hunt-Hoppert rats, that the fissures of the lower molar teeth in the caries-susceptible rats were wider than corresponding fissures in caries-resistant rats. They did not believe that fissure width was one of the factors involved in initiation of caries. Lactobacilli have been recovered in higher concentrations from the mouths of caries-susceptible than from caries-resistant rats of the Hunt-Hoppert strains.[6-9] Keller, Hunt, and Hoppert[10] suggested that the inherited difference between the 2 Hunt-Hoppert strains was not related to a substance or substances present in the secretions of the parotid gland, since removal of the parotid glands or excision and ligation of the parotid ducts did not cause any earlier onset of macroscopic carious lesions in rats from either the caries-susceptible or caries-resistant strains during maintenance on the Hoppert-Webber-Canniff coarse particle diet. However, Schwartz, Resnick, and Shaw[11] have observed that the Hunt-Hoppert caries-resistant rats have an increased caries incidence if parotid gland removal or parotid duct ligation and excision is followed by maintenance on a purified cariogenic dietary regimen.

Sreebny, Meyer, and Bachem[12] observed that the protease activity of the submaxillary glands in male Hunt-Hoppert rats of the caries-susceptible strain was five times that of the caries-resistant strain. The intralobular ducts were thought to be the site of protease activity as a greater frequency of ducts was observed in the caries-susceptible strain. Amylase activity of the glands was the same in both strains. Willett, Rosen, Stafseth, Hunt, and Hoppert[13] extended the observations on protease activity to stimulated saliva from both males and females, and found that a similar difference existed between the

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saliva samples of the Hunt-Hoppert strains. However, protease determinations in the Harvard caries-resistant and caries-susceptible strains did not indicate that there was any correlation between caries-susceptibility and salivary protease activity in the Harvard strains.[14]

Rosen, Benarde, Fabian, Hunt, and Hoppert[15] studied the following eight properties of saliva from Hunt-Hoppert rats: pH, amylase activity, relative viscosity, surface tension, refractive index, specific gravity, agglutinin titer for rat oral lactobacilli, and antagonistic effect of saliva against rat oral lactobacilli. The relative viscosity was possibly significantly higher in saliva from caries-resistant rats but no differences were observed in the other seven parameters.

None of the above parameters appear likely to bear a causative relationship to dental caries. Several systemic conditions have been shown to alter the initiation and progress of carious lesions after enamel formation. Gonadectomy[16] and hyperthyroidism[17] caused decreased caries incidence in rats, while hypothyroidism,[17] hypophysectomy,[18] and estrogen administration[19] were responsible for increased caries incidences. Gonadectomy decreased caries incidence in male hamsters.[20]

In view of these varied demonstrations of a relationship between systemic conditions and dental caries incidence, the present study on the parabiosis of caries-susceptible and caries-resistant rats was performed in an effort to determine whether an inherited difference in caries susceptibility may be in part due to a factor or factors carried in the blood stream.

EXPERIMENTAL

Two colonies of caries-susceptible and caries-resistant albino rats were used. One colony was developed at Harvard, and consists of two susceptible strains and one resistant strain. The other colony was developed at Michigan State University and consists of the Hunt-Hoppert susceptible and resistant strains. Both colonies are maintained routinely in the Harvard laboratories on a purified cariogenic diet[21] throughout the complete reproductive cycle, for the production of standardized offspring to be used in experiments concerning dental caries.

At about 21 days of age, parabiosis was performed according to the method of Bunster and Meyer.[22] The operations were achieved under ether anesthesia and clean, but nonsterile, conditions.

The rats were grouped and paired with respect to strain, age, weight, and sex. Parabiosis was performed on a total of 165 pairs of rats with the operative pairing into 3 distinct groups: resistant-susceptible pairs, resistant-resistant pairs, and susceptible-susceptible pairs. The latter 2 groups were formed by pairing within litters. The resistant-susceptible group was formed by pairing closely matched rats from two litters that were representative of their respective strains. A difference of more than 1 day of age or 5 grams in weight between individuals of any resistant-susceptible pair was rare. In all instances, some animals from each litter were kept as unoperated controls under otherwise identical dietary and environmental circumstances for a later comparison of their caries incidences with those of their parabiont littermates.

The parabiotic pairs and their unoperated littermates were fed a cariogenic diet[21] ad libitum, from weaning at 21 days until the end of the experiment. The original Harvard cariogenic diet had been modified by the substitution of crude casein for purified casein, and by the addition of 15 per cent Cellu flour.

The resistant-resistant pairs were sacrificed between 206 and 210 days of age, a time when grossly visible carious lesions were beginning to appear in the resistant rats. The resistant-susceptible and susceptible-susceptible pairs were sacrificed at 102 days of age, a time when grossly visible carious lesions were beginning to occur in the representatives of the susceptible strain. Four pairs of rats which separated between 90 and 100 days of age were included in the average for the resistant-susceptible group. It is assumed that a circulatory exchange existed until the time of separation. This is a reasonable assumption since granulation-like tissue persisted in the wound site. Further, any effect that 1 rat of a pair would have had on the other would probably be evident by the time of separation. Three pairs of resistant-susceptible rats were not killed until 207 days of age. Two of the latter pairs were not included in the table on group comparison of caries activity, since their unoperated littermate controls had died. The third pair and their littermate controls are included in the average despite the longer experimental period.

Trypan blue was selected as a potentially suitable agent for determination of the extent of blood interchange between animals of a pair because of the following properties: (1) its presence in the blood stream is grossly evident, (2) it is a colloidal dye tending to form aggregates, and (3) it can be used safely at dosages several times those used in this experiment.[23] A 1 per cent aqueous solution of trypan blue was filtered twice through No. 1 Whatman paper. Under ether anesthesia, the rats received femoral vein injections of 5 mg. of dye per 100 grams of body weight.

Blood samples, for determination of serum trypan blue levels, were collected at the time of death from the abdominal aorta. After centrifugation, measurements of the trypan blue concentration were determined with a Coleman Junior spectrophotometer[23] at a wave length of 595 mμ. The animals were killed by ether and decapitated. The heads were kept in 95 per cent ethanol for caries evaluation by previously established criteria.[24] Sections of the liver were fixed in Susa solution[25] and stained with eosin to contrast with the trypan blue particles and the parenchymal cells.

RESULTS

A total of 165 operations was performed. Fourteen pairs of rats were not included in this study for such reasons as the death of one or both rats during operation, or constant postoperative fighting to the extent that the pair had to be killed prior to the fulfillment of any useful experimental period. Of 40 parabiotic littermate pairs, 15 (37 per cent) died and 1 (3 per cent) separated, a total of 16 (40 per cent) unusable pairs. Of 111 parabiotic nonlittermate pairs, 69 (62 per cent) died and 37 (33 per cent) separated, a total

of 106 (95 per cent) unusable pairs. Both the rate of death and the rate of separation were appreciably higher for non-littermate pairs than for littermate pairs.

The length of time after operation at which 15 littermate and 69 non-littermate pairs died and 1 littermate and 37 non-littermate pairs separated is shown in Fig. 1. The greatest number of deaths occurred in the first post-operative week and decreased in each succeeding week thereafter to a negligible value by the sixth week. The rate of separations increased to a maximum during the sixth week, after which a decrease was observed.

In general, the non-littermate pairs did not appear as healthy as the littermate pairs at any time during the experimental period. The regrowth of fur near the wound site was sparser and rougher in non-littermate parabionts and there was a persistence of granulation-like tissue in the wounds. The separated pairs were always those in which the cicatrix was more poorly formed.

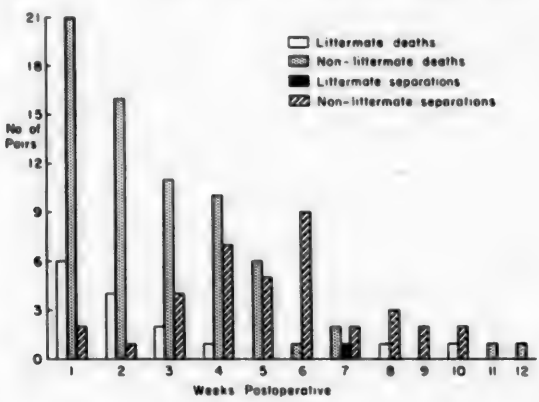

Fig. 1.—Death and separation rates among parabionts.

Seventeen littermate and non-littermate pairs of rats were used in the trypan blue injection studies on cross circulation. There were no apparent ill effects following injection of any pair of rats. The injected rat became a definite blue color immediately after introduction of the dye into the blood stream. The uninjected parabiont began to show the first signs of the dye about 45 minutes later and by the end of 4 hours was a definite blue color, although somewhat less intense than that which the injected parabiont exhibited. These color differences in the 2 rats relative to time were distinct and pronounced.

Measurements of serum trypan blue levels were made in 3 littermate pairs of rats just prior to the end of the usual experimental period. The serum trypan blue levels for both animals of a pair 4 hours, 2 days, and 3 days postinjection were approximately the same in the 2 parabionts at each time interval.

If trypan blue was diffusing from 1 parabiont to the other, outside existing vascular channels, one would expect particulate matter to be found in the reticuloendothelial system of the injected rat only. However, typical aggregates of trypan blue particles were found in the Kupffer cells of the livers of

both animals of any pair, sacrificed after injection of one member with trypan blue. The number of particles per unit area was approximately the same in the livers from both rats of a pair.

The vascular anastomoses between the parabionts are capillaries and would be expected to develop in the wound area on about the fourth or fifth day. On the second postoperative day, 1 parabiont of a non-littermate pair was injected with trypan blue. On the third postoperative day, only the injected rat was blue. By the sixth postoperative day, both rats were a similar shade. In another non-littermate pair, in which the skin suture line had separated leaving only the abdominal muscles connecting the 2 rats, a trypan blue injection was made to determine whether any appreciable degree of capillary anastomoses existed. On the fourth postinjection day, the line of scar formation served as a distinct marker, separating the blue muscle of the injected rat from the red muscle of the uninjected parabiont. One of the 3 pairs of 6-month-old non-littermate parabionts that was injected with trypan blue showed signs of impaired cross circulation. The uninjected animal did not turn a definite shade of blue until 12 hours postinjection.

The average caries scores of the operated and intact rats of the resistant-resistant, susceptible-susceptible, and resistant-susceptible groups are presented in Table I. In all cases, the average number of carious teeth, the average number of carious lesions and the average extent of carious lesions for any group of operated rats were not significantly different from the average caries scores of their control littermates. For example, within the resistant-susceptible group, the average caries incidences of the operated resistant rats did not significantly differ from the average caries score of the control resistant littermates, and the average caries score of the operated susceptible rats did not significantly differ from the average caries score of the control susceptible littermates. It can be seen that a similar comparison existed between the average caries scores of the operated and control rats within the resistant-resistant and susceptible-susceptible groups.

TABLE I

A COMPARISON OF THE DENTAL CARIES EXPERIENCE AMONG PARABIOTIC RATS AND THEIR UNOPERATED LITTERMATES

STRAIN PAIRING	CONDITION	NO. OF RATS	DURATION OF EXPERIMENT (DAYS)	AVERAGE NUMBER OF CARIOUS MOLARS	AVERAGE NUMBER OF CARIOUS LESIONS	AVERAGE EXTENT OF CARIOUS LESIONS
Resistant-resistant	Parabiont	22	206-210	3.2	5.0	10.2+
	Control	10	206-210	3.2	4.5	11.6+
Susceptible-susceptible	Parabiont	26	102	8.6	15.8	48.4+
	Control	16	102	8.1	13.1	39.1+
Resistant-susceptible	Resistant parabiont	7	102	2.3	4.1	8.5+
	Resistant control	9	102	2.9	4.7	7.1+
	Susceptible parabiont	7	102	8.9	15.0	35.3+
	Susceptible control	12	102	7.8	11.7	35.0+

DISCUSSION

The immune mechanism appears to be responsible for several phenomena observed in parabionts, sometimes referred to collectively as parabiosis intoxication. The hypothesis that there is likely to be a greater chance of successful parabiosis among littermate rats than among non-littermates because of greater similarity of antigenic constitutions within litters is supported by a comparison of our mortality rates among non-littermate and littermate pairs. The non-littermate pairs had a higher percentage of deaths throughout the experimental period. Although littermate pairs died throughout the same period, the rate of deaths was consistently lower.

Immune reactions may manifest themselves in numerous ways. Instances may occur in which a pair of rats is matched well enough antigenically for survival, but not well enough to prevent the occurrence of certain consequences as decreased rate of blood exchange, absence of vascular anastomoses across the region of muscle fusion, poor general health and separation. All or many of these latter phenomena were noted among non-littermate pairs.

Finerty and Panos[26] studied parabiosis intoxication in male albino rats of the Holtzman-Rolfsmeyer strain. At the end of a 7-week period, they reported a 64 per cent mortality in 78 non-littermate pairs and a 50 per cent mortality in 10 littermate pairs, but did not mention the frequency of separation nor the health of their survivors. If no problems of separation or poorer health were encountered among their non-littermate pairs, they obtained a much higher survival of non-littermates than we did. The authors stated further that, after 3 weeks, only rarely did a pair of rats die. In this present study, roughly 25 per cent of the pairs died after reaching the age of 3 weeks. The over-all difference in results may have been due to the fact that their non-littermates were from the same strain, and hence more nearly similar to each other antigenically than our non-littermates which were representatives of separate and genetically different strains.

Hill[27] has found that the rate of exchange varied from pair to pair of parabionts as well as within a pair when measured on separate occasions. No effort was made in this study to detect slight differences in the rate of exchange or to make more than one determination in a pair. However, distinct differences between non-littermate pairs were observed. If less pronounced differences were present in littermate pairs, they were not noted.

In some hormone experiments with parabiotic rats, there appeared to be a "parabiotic" barrier to the transfer of certain hormones, i.e., in a pair of parabiotic rats in which a specific hormone concentration was absent or greatly diminished in one animal, the hormone of the other animal failed to exert its effect on its partner, yet a different hormone in a similar situation would affect both parabionts readily. Hill's studies,[27,28] with the dye brilliant vital red, clarified this puzzling finding. He concluded that the height of the level in the parasite was related directly to the rate of blood exchange and inversely to the speed of absorption and elimination of the hormone by the tissue. Thus, a substance in one parabiont that is excreted or metabolized rapidly may never reach

a sufficiently high concentration to affect the other parabiont because of the relatively slow rate of blood exchange. Hill's conclusions were supported by Grollman and Rule[29] and by Huff, Trautman, and Van Dyke.[30] Accordingly, the lack of difference in caries scores between operated rats and intact controls of the resistant-susceptible group does not completely rule out the possibility that a blood-borne factor or factors influences caries incidence in the resistant and/or susceptible animals.

Certainly one of the biggest limitations of the parabiotic technic lies in the fact that negative results cannot be considered conclusive. However, the results of this study do indicate that the intimate relationship which parabiosis permits has not overriden the genetic predisposition of 1 parabiont to be reasonably caries-susceptible while his companion is comparably caries-resistant. For additional studies, a method of establishing a rapid interchange of blood between parabionts is needed to determine whether these negative results were significant or whether there was a blood-borne factor that we have failed to detect.

SUMMARY

1. The mortality rate of littermate and non-littermate pairs was 37 per cent and 62 per cent, respectively. An additional 3 per cent of the littermate pairs and 33 per cent of the non-littermate pairs were lost due to separation. The non-littermate pairs healed less satisfactorily and looked less healthy. The percentage of useful pairs obtained was 60 per cent for littermates and 5 per cent for non-littermates.

2. The rate of blood interchange among the non-littermate pairs varied considerably. Vascular channels did not develop across the muscle fusion in at least 1 pair of non-littermate rats.

3. After injection of trypan blue into 1 parabiont, the gross color changes in the uninjected parabiont during the following several hours appeared to be reliable criteria for estimation of the effectiveness of circulatory exchange. This technic was simple to execute, easy to read, and did not noticeably affect the health or future usefulness of the animals.

4. Parabiosis, per se, did not alter caries activity among caries-susceptible and caries-resistant rats. The failure of a caries-resistant parabiont to cause any modification in the caries initiation and progression of his caries-susceptible parabiont, and vice versa, does not mean that these negative results conclusively eliminate the possibility of a blood-borne determinant of the degree of proneness to caries.

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PRODUCTION OF CLINICAL ROENTGENOGRAMS BY MEANS OF COMPACT RADIOACTIVE X-RAY AND GAMMA-RAY SOURCES

An Experimental Study

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I. BRIEF REVIEW OF THE LITERATURE

ARGON of mass 37, which has a half-life of 34 days, was one of the first radioactive isotopes to be discovered, which emitted x-rays only during the process of decay.[1] Since 1941, many additional radioactive isotopes have been discovered which emit x-rays. A brief review of some 200 isotopes, distinguished by their ability to decay in such a way that the emission of characteristic x-rays resulted, was published in 1949.[2] In more recent years, additional x-ray emitting radioactive isotopes as well as isotopes, which emit low-energy gamma rays in the normal x-ray region of the electromagnetic spectrum, have been found subsequent to cyclotron or nuclear reactor irradiation of certain stable isotopes.

The quality of radiographs produced by electromagnetic radiations from radioactive substances and from x-ray machines is dependent upon wave length when other factors remain constant. Most commonly used commercial dental x-ray machines are said by their manufacturers to operate at an energy of 65 kv. There is, at present, a growing tendency to use somewhat higher energy x-rays in both the field of dental and medical roentgenography.[3, 4, 5]

Cesium^{131} is a radioactive isotope which emits no gamma rays but only the characteristic x-rays of xenon. Satisfactory dental roentgenograms were produced by radiations from this isotope in 1948.[6, 7] The disadvantage of Cs^{131} is its short half-life of 10.0 days. The fact that this isotope can be made carrier free (and thus the specific activity of this source can be nearly 100 per cent) is a real advantage.

In 1952, thulium^{170} which emits a low-energy gamma ray of 84 kev, was used as a source to radiograph a living human hand.[8] The exposure time was 5 minutes. In 1954, 3 reports were published on Tm^{170} as a source of radiations for clinical radiography.[9, 10, 11] In 1956, the use of radiations from Tm^{170} for exposure of film during kidney surgery produced satisfactory radiographs.[12] The portable unit housing the isotope made possible views heretofore not possible.

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Americium^{241} has been used as a source of radiations. Radiographs of a femur were demonstrated in 1953.[13] The use of bremsstrahlung from strontium^{90}—yttrium^{90} for exposure of radiographs was demonstrated in 1954[14] and 1955.[15]

In 1954, a review of the literature on the use of radiations from radioactive isotopes for exposure of film for medical radiographic purposes was published.[16] The use of radiations from cerium^{144} as a source was demonstrated.

The high energy radiations from radium and cobalt^{60} have been used for many years to produce radiographs of heavy metallic structures. The problem of nondestructive testing of aircraft components by means of radiography using radiations from europium^{155}, cerium^{144}, americium^{241}, iridium^{192}, and thulium^{170} was investigated.[17] Of these isotopes, Ir^{192} and Tm^{170} were selected as being more satisfactory than the others. It was found that some types of flaws could be detected without the need of excessive exposure time.

II. MATERIALS AND METHODS

Exposure Techniques.—A no-screen type emulsion was used in the production of the radiographs illustrated in Fig. 2 and in Fig. 6. The sources were in a lead cubic cylinder approximately 1 inch in diameter. The skull, penetrometers, and extracted tooth were placed on a film holder. The source film distance was 16 inches. The radiograph shown in Fig. 2 received 0.4 roentgens and that in Fig. 6, 0.06 roentgens. The 2 different sources were of such intensities that the respective exposure times were 20 hours and 18 hours. Radiographs produced by the strongest Ce^{144} source available to us required exposure times of approximately 10 minutes.

Costs.—The cost of radioactive Ce^{144}, processed and carrier-free, is $1.00 per millicurie. The cost of radioactive Cs^{137} is 10 cents per millicurie and $25.00 per curie in excess of one curie. Stable samarium^{144} costs $8.00 per milligram when enriched from 3.16 per cent to 85.7 per cent. Samarium^{154} enriched to 99.1 per cent, however, costs only 70 cents per milligram. The stable isotopes must be irradiated in a reactor at a charge rate per week of $50.00 or more. The prices of the above items and also of others are tending to decrease.

III. REPORT OF ISOTOPES INVESTIGATED

A. *Cerium^{144}.*—Ce^{144} is one of the fission products obtained from the "ash" of a nuclear reactor. Ce^{144} decays with a half-life of 290 days to praseodymium^{144} which in turn decays with a half-life of 17.5 minutes to "stable" neodymium. The transformation from Ce^{144} to Pr^{144} gives rise to useful radiations (Fig. 1) in the 34, 81, and 134 kev regions. In 5 per cent of the transitions from Pr^{144} to Nd^{144}, very high-energy gamma rays are emitted (Fig. 1). The spectrum was measured with a single channel pulse height analyzer. The intensity of the energies of the gamma rays in the high-energy region differed in 3 different samples of Ce^{144} which were investigated. The reason for this finding is not clearly understood. In order to reduce exposure times for the production of radiographs, a "one curie source" was obtained from Oak Ridge.

This source was prepared from material which was about 8 years old. Consequently, the source was found to be more bulky than had been expected. Furthermore, unlike the spectrum of the minute sample shown in Fig. 1, the spectrum of a minute sample from the "one curie source" was very prominent in the intensities of the radiations in the high energy region.

In spite of the radiations in the high-energy region, very satisfactory radiographs were produced by radiations from Ce^{144} (Fig. 2). The contrast of the film exposed by radiations from Ce^{144} was considerably less than the contrast of the film exposed by radiations from the conventional x-ray machine. There was good detail in the region of the petrous portion of the temporal bone as well as of the ascending ramus of the mandible when radiations from Ce^{144} were used. Both regions could not be visualized on the same film at the same time

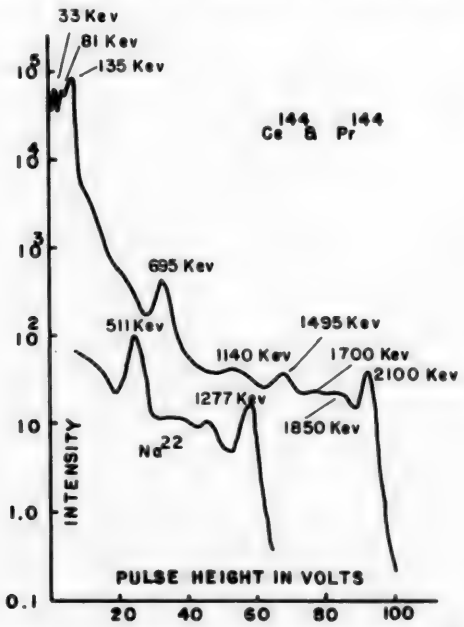


Fig. 1.—Scintillation spectrum of Ce^{144}-Pr^{144} showing strong low-energy radiations and weak high-energy radiations.

when x-ray radiations from a commercial dental x-ray machine were used in spite of variation in both time and kilovoltage. Furthermore, with Ce^{144} radiations, the anatomic details which are usually obscured by the shadow of the base of the skull and the petrous portion of the temporal bone, were clearly visualized. The details of the pulp chamber and other structures of the extracted tooth were visualized very satisfactorily.

If a fresh Ce^{144} sample, chemically separated very recently from fission products, becomes available and emits a spectrum similar to that shown in Fig. 1, Ce^{144} would be an outstanding contender as one of the good radioisotopic sources for clinical radiography.

B. *Europium*^{155}.—Europium^{155} has a number of properties which make this radioactive nucleus satisfactory for low-energy isotopic radiography. Eu^{155} can be prepared in carrier-free form from neutron bombardment of samarium^{154} which is one of the 6 stable isotopes of samarium. The energies of the radiations emitted by Eu^{155} are 18, 42, 84, and 102 kev. The 84 kev radiation is the most intense. The 42 kev radiation represents the x-rays of gadolinium from internal

Fig. 2.—Radiograph of skull, penetrometers, and an extracted tooth made by exposure to radiations from Ce^{144}-Pr^{144}.

conversion and are nearly as intense as the 84 kev radiation. The weakness of the 18 and 102 kev radiations makes their observation on a crystal spectrometer difficult. In addition to these radiations, weak radiations extend from 225 kev to 1.32 mev region. The complete spectrum is shown in Fig. 3.

The eventual clinical use of Eu^{155} radiations will depend upon the removal of the weak high-energy radiations which arise from impurities. These are primarily Eu^{152}, Eu^{154}, and Eu^{156}. These impurities cannot be removed chemically from Eu^{155}. If Eu^{155} plus these impurities were put through a calutron, a separation could be affected.

We have found that the half-life of Eu^{155} is approximately 5.5 years instead of the 1.7 years reported in the literature.

C. *Samarium*^{145}.—This isotope is prepared by irradiating Sm^{144} with neutrons. Sm^{145} has a half-life of 1.12 years and decays to promethium^{145} which in turn is transformed by electron capture, with a half-life of 30 years, into

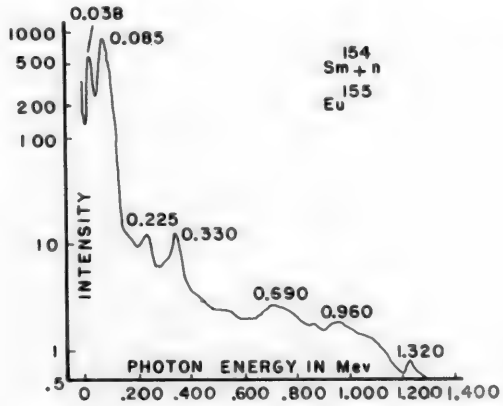


Fig. 3.—Scintillation spectrum of Eu^{155}.

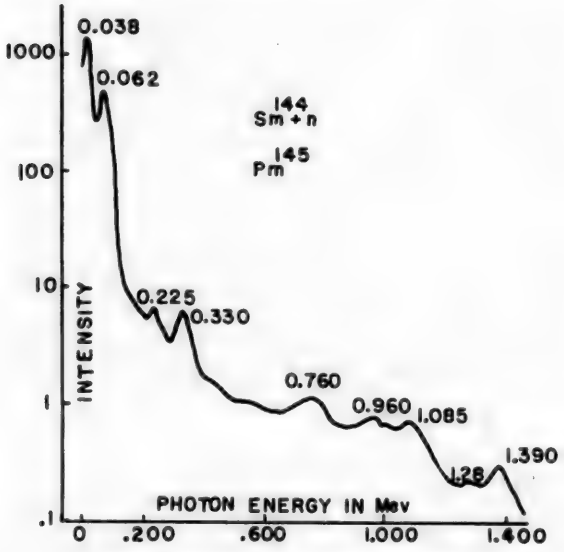
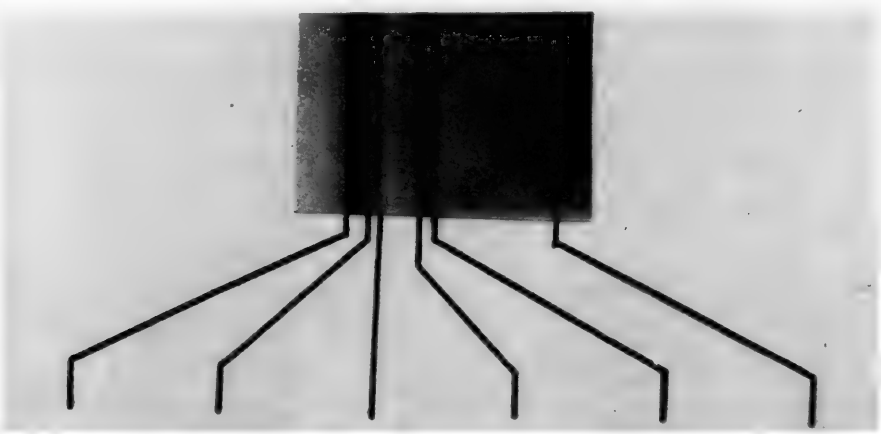


Fig. 4.—Scintillation spectrum of Sm^{145}.

stable Nd^{145}. The scintillation spectrometer and also the Cauchois spectrograph show that Sm^{145} emits weak gamma rays of 62 kev and a very strong promethium x-ray of 38 kev. No other radiations are observed from this radioactive isotope. Impurities which are present are manifested by the several high-energy gamma

rays which are shown in the scintillation spectrometer graph (Fig. 4). These impurities are Eu^{152}, ^{154}, and ^{156} and can be completely removed by an ion exchange column. The purified Sm^{145} source produced a spectrum similar to Fig. 4 with the exception that no energies were found higher than 62 kev. Sm^{145} is exceptionally well suited for low-energy roentgenography. The spectrum as obtained by a Cauchois spectrograph is shown in Fig. 5. The Kα lines are the blackest ones on the left side of the print.

A radiograph using radiations from Sm^{145} was made of a skull, aluminum step wedges, and an extracted tooth (Fig. 6). This radiograph has more contrast than the radiograph exposed by radiations from Ce^{144} but less than the contrast of roentgenograms exposed by radiations from a commercial x-ray machine.



Pm	Pm	Sm	Pm	Pm	Gamma ray
$K\alpha_2$	$K\alpha_1$	$K\alpha_1$	$K\beta_3$	$K\beta_2$	from Sm^{145}
38.15 kev	38.62 kev	39.95 kev	43.6 kev	45.0 kev	61.3 kev

Fig. 5.—Cauchois spectrogram of Pm x-rays and gamma ray from Sm^{145}.

D. *Lanthanum*^{137}.—La 137 has a very desirable spectrum for low-energy roentgenography. The spectrum consists of a single x-ray radiation of 31 kev. No gamma-rays are emitted. The shielding problem is, therefore, at a minimum. La^{137} which can be produced from Ce^{136} plus neutrons, is carrier free with a specific activity approaching 100 per cent. Unfortunately, La^{137} has a half-life of 6×10^5 years, a fact that was not previously known. The long half-life results in the intensity of the activity being so low that this isotope is restricted to academic experiments.

E. *Dysprosium*^{157}.—Two of the 4 radioactive isotopes of Dy are not well known. Dy^{157} is of class "B," has a half-life of 8.2 hours and decays into terbium^{157} about which nothing is known. Attempts to detect radioactive Tb^{157} were unsuccessful because of numerous other strong activities arising from the several isotopes of Dy and from impurities.

Fig. 6.—Radiograph of skull, extracted tooth, penetrometers, and lead block produced by radiations from Sm^{145}.

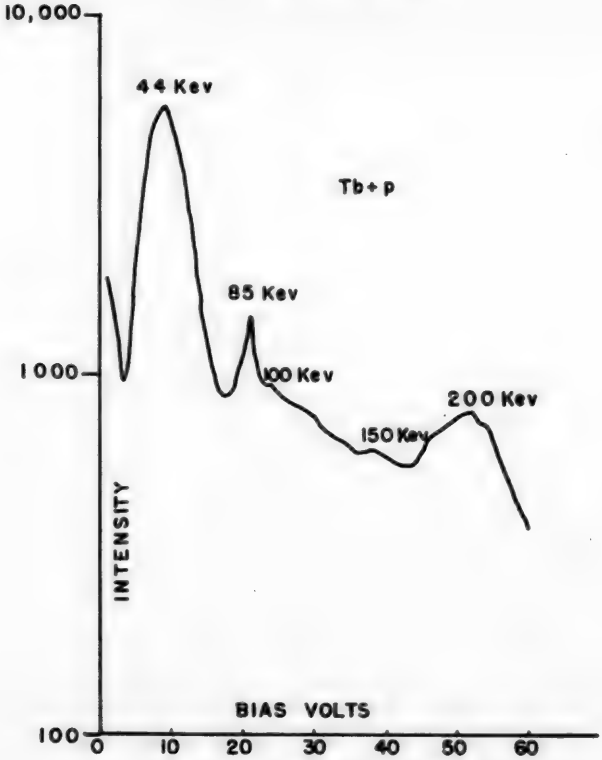


Fig. 7.—Dy^{159} spectrum from Tb + p.

F. *Dysprosium*[159].—Dy^{159} has a half-life of 134 days and emits terbium x-rays but no undesirable high-energy gamma rays. Dy is now being made available in the enriched form at Oak Ridge. Dy^{159}, obtained from enriched Dy^{158} plus neutrons, offers possibilities as a desirable source for low-energy radiography. The spectrum shown in Fig. 7 was obtained by use of the customary crystal spectrometer technic. The activity was produced by proton bombardment of the single stable isotope of terbium, viz., Tb^{159}.

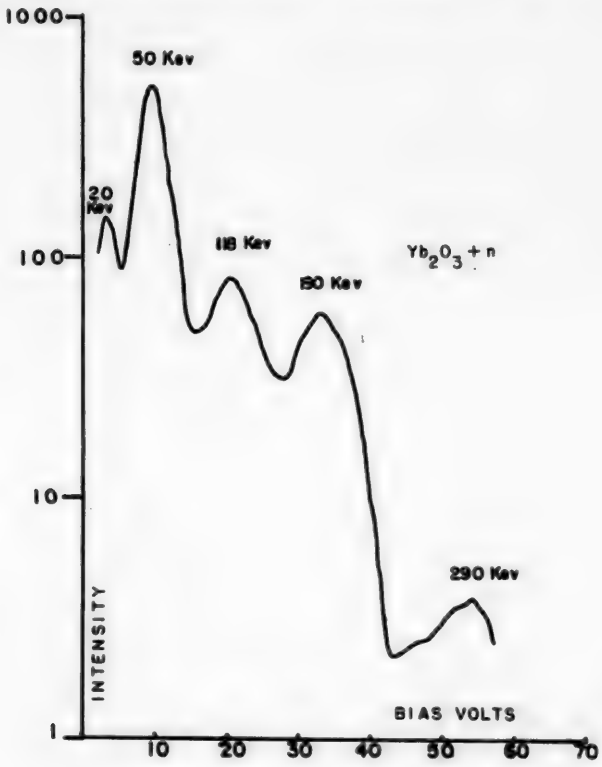


Fig. 8.—Scintillation spectrum of Yb^{169}.

G. *Ytterbium*[169].—The spectrum of Yb^{169} is shown in Fig. 8. A strong radiation is observed at 50 kev. Penetrometer radiographs were made to confirm the good quality of clinical radiograph expected from this radioactive source. When enriched Yb^{168} becomes available, Yb^{169} could well be a very important isotope for radiographic purposes because of 3 factors. First, the half-life of 32.5 days is relatively short which results in high intensity. Second, the neutron cross section of 11,000 is exceptionally large which again results in high intensity. Third, the emitted radiations are in the desirable range of energies.

H. *Gadolinium*[153].—The spectrum of Gd^{153} is shown in Fig. 9. The emissions from this isotope are nearly ideal. There is a strong 41 kev x-ray and a strong 102 kev gamma ray. The 144 kev peak shown in the spectrum is caused

by the summing of the 41 kev and 102 kev radiations in the crystal. Thus, there is no 144 kev emission. A very weak 71 kev radiation is present. The 625 kev radiation of extreme weakness is due to an impurity. The radioactive material, the analysis of which is illustrated in Fig. 9, was not subjected to chemical separation.

I. *Other Sources Studied.*—The radioactive nuclei platinium[193], gold[199], ruthenium-technetium[97], tungsten[175], and erbium-holmium[163] do not seem to offer useful possibilities in the field of low-energy radiography.

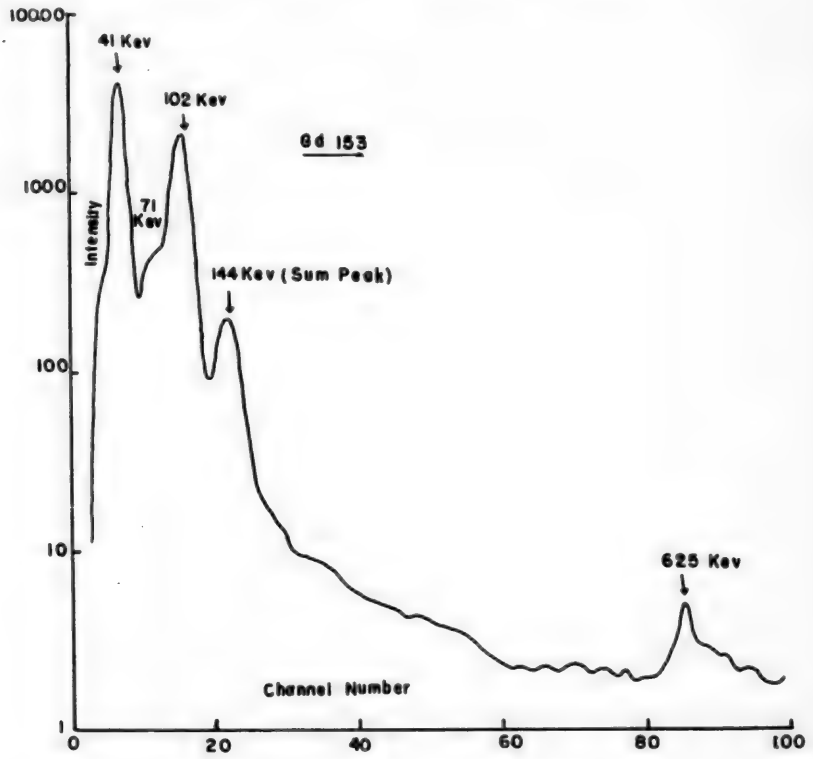


Fig. 9.—Scintillation spectrum of Gd^{153}.

IV. CONCLUSIONS

1. Radiations from radioactive sources produce excellent quality radiographs even though in some instances the energies emitted are considerably higher than those emitted by conventional x-ray equipment.

2. The single radioactive source for a wide range of purposes in the low energy region (30 to 150 kev), with the best radiation characteristics, with a convenient method of production and free from impurities cannot be designated at this time.

3. There are several radioactive isotopes which, if freed from impurities and prepared properly, have the characteristics which are desired in a low-energy, high intensity source for radiographic purposes.

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A COMPARISON OF THE SALIVARY PROTEASE ACTIVITY IN THE HARVARD AND HUNT-HOPPERT CARIES-RESISTANT AND CARIES-SUSCEPTIBLE RATS

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DURING the past two decades, two groups of investigators have worked independently on the development of genetically caries-susceptible and caries-resistant strains of laboratory rats: the Hunt-Hoppert strains at Michigan State University and the Harvard strains at the Harvard School of Dental Medicine. There is a distinct advantage in the use of the offspring from these strains in the study of tooth decay, because the colonies provide subjects of known genetic constitution and of known caries traits for controlled investigative procedures. Thus, in any experimental regimen, the equal littermate distribution of offspring from these strains among the control and experimental groups makes possible a greater degree of control from group to group and from experiment to experiment than is possible with rats from a less well-defined hereditary background.

Since saliva is normally in continuous contact with the teeth, predisposing influences to either resistance or susceptibility in these rodents may be related to certain physical, microbiologic, or biochemical differences of the saliva of these strains. The saliva of mature Hunt-Hoppert caries-susceptible rats has been shown by the Michigan State investigators to possess three times as much protease activity as the saliva of comparable caries-resistant animals.[1] The present studies were undertaken to determine if similar differences existed between representative groups of rats from the caries-susceptible and caries-resistant strains that had been developed in the Harvard laboratories. A second objective was to determine whether a correlation existed between salivary protease activity and the exact caries score of the representatives within each strain. To our knowledge, the latter procedure was not applied to the representatives of the Hunt-Hoppert strains in the earlier studies. In addition, the salivary protease values of representatives of the Hunt-Hoppert strains were evaluated under the experimental conditions in our laboratories as a means to determine whether the different laboratory and environmental factors had had any influence on salivary protease values.

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METHODS

The rats used in the first 2 experiments were representatives of the Harvard caries-resistant and caries-susceptible strains, the breeding colonies of which are maintained throughout their life histories on a purified cariogenic diet, 700 + 15 per cent Cellu flour.[2] The offspring used in these studies likewise were maintained on the same diet. In contrast, the Hunt-Hoppert rats in the published studies from Michigan State University had been maintained on the Hoppert-Webber-Canniff coarse particle diet both during reproduction and during the experimental periods when saliva samples were being collected.[1]

No attempt was made to select closely related subjects within either strain as a reasonably wide representation of the 2 strains was sought. The number of males and females in each strain in the 2 experiments is given in parentheses below the protease and protein values in Table I.

Saliva was collected by the method of Benarde, Fabian, Rosen, Hoppert, and Hunt,[3] which consists of Nembutal anesthesia followed by pilocarpine stimulation at a dosage of 7.5 mg. per kilogram of body weight. Prior to injection with pilocarpine, the teeth had been vigorously scrubbed with a small brush dipped in distilled water to reduce contamination by fecal matter, sawdust, and retained food particles. However, it is realized that complete prevention of contamination from these sources is not feasible. An average of 1.5 ml. of saliva was collected per rat.

Individual whole saliva samples from a total of 77 rats in 2 separate experiments were tested for protease activity and protein content. In the first experiment, the animals were tested at approximately 110 and 180 days of age, while in the second experiment the animals were tested at approximately 60, 110, and 180 days of age. The 2 experiments were otherwise identical. The second experiment was conducted in order to confirm the results of the first, in view of the fact that the results were at variance with those from earlier experiments by the Michigan investigators.[1]

Protease activity was estimated after incubation for 4 hours at 38° C. of 0.5 ml. of saliva with 0.5 ml. of a 1 per cent solution of casein (Hammersten quality) in 0.2 M borate buffer at pH 9.6. The undigested casein was precipitated with 5 per cent trichloracetic acid and the concentration of tyrosine and tryptophane and related compounds in the supernatant liquid determined colorimetrically after color development with Folin's phenol reagent.[4-6] Results were expressed as micrograms of tyrosine and its equivalents liberated per milliliter of saliva.

Total protein content was determined on an aliquot of each sample of saliva in order to provide a potential base line for enzyme activity. Determinations were carried out colorimetrically using Folin's phenol reagent,[4] according to the method of Lowry, Rosebrough, Farr, and Randall.[7]

At approximately 200 days of age, the rats in the Harvard caries-resistant and caries-susceptible strains were sacrificed and their caries scores determined

TABLE I

PROTEASE ACTIVITY AND PROTEIN CONTENT IN STIMULATED WHOLE SALIVA FROM HARVARD AND HUNT-HOPPERT CARIES-SUSCEPTIBLE AND CARIES-RESISTANT RATS*

AGE IN DAYS	RESISTANT MALE		RESISTANT FEMALE		SUSCEPTIBLE MALE		SUSCEPTIBLE FEMALE	
	PROTEASE ACTIVITY†	PROTEIN CONTENT‡	PROTEASE ACTIVITY†	PROTEIN CONTENT‡	PROTEASE ACTIVITY†	PROTEIN CONTENT‡	PROTEASE ACTIVITY†	PROTEIN CONTENT‡
Experiment 1—Harvard strains on ration 700 + 15% Cellu flour								
110-128	180 ± 50 (8)	6.0 ± 0.9 (8)	120 ± 10 (10)	5.8 ± 0.7 (10)	120 ± 10 (13)	5.7 ± 0.6 (13)	80 ± 10 (6)	6.3 ± 0.6 (6)
182-196	140 ± 20 (6)	5.8 ± 1.2 (6)	120 ± 20 (10)	6.1 ± 0.4 (10)	200 ± 40 (13)	5.0 ± 0.5 (13)	110 ± 10 (15)	7.6 ± 0.6 (15)
Experiment 2—Harvard strains on ration 700 + 15% Cellu flour								
61-67	160 ± 20 (4)	6.3 ± 1.2 (4)	120 ± 30 (5)	7.0 ± 0.5 (5)	90 ± 10 (12)	5.2 ± 0.4 (12)	110 ± 20 (9)	5.2 ± 0.4 (9)
100-110	240 ± 100 (3)	5.4 ± 0.8 (3)	170 ± 50 (5)	5.7 ± 0.7 (5)	140 ± 30 (12)	5.7 ± 0.6 (12)	150 ± 10 (7)	5.4 ± 0.5 (7)
180-190	140 ± 20 (3)	7.1 ± 1.9 (3)	350 ± 130 (5)	9.1 ± 1.8 (5)	270 ± 50 (13)	7.6 ± 0.8 (13)	210 ± 30 (7)	5.0 ± 0.6 (7)
Experiment 3a—Hunt-Hoppert strains on ration 700 + 15% Cellu flour								
60-68	140 ± 20 (7)	6.0 ± 0.7 (7)	110 ± 20 (5)	6.7 ± 0.8 (5)	300 ± 20 (16)	6.1 ± 0.5 (16)	220 ± 20 (14)	6.5 ± 0.6 (14)
85-95	110 ± 30 (4)	4.1 ± 0.6 (7)	120 ± 30 (2)	3.1 ± 0.9 (2)	410 ± 40 (16)	4.9 ± 0.4 (16)	270 ± 30 (11)	6.2 ± 0.7 (11)
Experiment 3b—Hunt-Hoppert strains on ration HWC								
60-68	130 ± 30 (4)	6.5 ± 0.8 (4)	80 ± 10 (7)	5.9 ± 0.7 (7)	-	-	-	-
85-95	80 ± 20 (3)	3.4 ± 0.7 (3)	90 ± 20 (3)	7.0 ± 1.2 (3)	-	-	-	-

*Average values and standard error of the mean with number of animals in each group in parentheses.
†Protease activity expressed as micrograms of tyrosine and equivalents liberated per milliliter of saliva.
‡Protein content of saliva expressed as milligrams of protein per milliliter of saliva.

by the method of Shaw, Schweigert, McIntire, Elvehjem, and Phillips.[8] Coefficients of correlation were calculated for each set of data to determine whether any significant correlation existed between protease activity and the caries score.

A third experiment was conducted with the Hunt-Hoppert caries-resistant and caries-susceptible strains maintained in our laboratories. This experiment was conducted in order to check the various methods, and to determine whether any difference in salivary protease activity between the Hunt-Hoppert strains could be detected for the colonies maintained under our laboratory conditions. Two separate parts made up the third experiment. In the first part (a), breeding pairs of the Hunt-Hoppert caries-resistant and caries-susceptible strains were maintained on the same purified cariogenic diet, 700 + 15 per cent Cellu flour,[2] used for maintenance of the Harvard colonies. The offspring used in this experiment were also maintained on the purified cariogenic diet. In the second part (b) of the experiment, breeding pairs of the Hunt-Hoppert strains were maintained on the Hoppert-Webber-Canniff (HWC) diet[9] that has been used in the laboratories at Michigan State University. The offspring of these matings were also maintained on the HWC diet. In this regard, part (b) of this experiment represented as close a duplication of the experimental circumstances used at Michigan State University as possible in our laboratory. The number of rats in the various groups are shown in parentheses in Table I. The rats in the third experiment were sacrificed at approximately 110 days of age.

RESULTS

The results on salivary protease activity in the first and second experiments with the Harvard strains of caries-resistant and caries-susceptible rats are shown in Table I. Throughout both experiments, a very high variability was observed in the individual salivary protease values for the animals within any group, and also in the average salivary protease values for the various groups. However, the salivary protease activity did not appear to be consistently higher in the rats from one strain than in the rats from the other strain. While certain differences between groups gave evidence of being statistically significant at the 1 per cent level, these differences were not consistent from sex to sex nor from experiment to experiment. Likewise, neither sex appeared to have consistently higher salivary protease values than the other sex. In the evaluation of all experimental data, good judgment based on knowledge of the nature and variability of the laboratory material, the methods employed, and the contingent circumstances must take precedence over statistical appraisals, valuable as they are as aids to good judgment. Despite the scattered differences that were indicative of statistical significance, the only tenable conclusion is that no consistent relationship existed between salivary protease activity and the caries-susceptibility of the Harvard strains. However, a definite trend toward higher salivary protease values with increasing age was observed among the caries-susceptible male and female rats. No comparable trend was noted with age among either sex of the caries-resistant strain.

TABLE II

AVERAGE SCORES FOR THE EXTENT OF CARIOUS LESIONS IN HARVARD AND HUNT-HOPPERT CARIES-RESISTANT AND CARIES-SUSCEPTIBLE RATS

EXPERIMENT NUMBER	STRAIN	DIETS*	RESISTANT MALES	RESISTANT FEMALES	SUSCEPTIBLE MALES	SUSCEPTIBLE FEMALES
1	Harvard	700	3.5+	2.7+	19.8+	36.0+
2	Harvard	700	5.0+	4.3+	56.0+	47.4+
3a	Hunt-Hoppert	700	10.7+	12.5+	29.8+	30.8+
3b	Hunt-Hoppert	HWC	25.5+	18.4+	-	-

*Ration 700 was routinely supplemented with 15 per cent Cellu flour.

The average scores for the extent of carious lesions for the various groups are shown in Table II. Since the rats in both Harvard strains had been sacrificed at 200 days, a good comparison can be made of the relative proneness to caries of the 2 strains as represented by the subjects in these experiments. In all groups, the caries-resistant rats on the average experienced a much lower incidence of dental caries than comparably treated rats from the caries-susceptible strain. Calculation of the coefficient of correlation between salivary protease activity and the extent of carious lesions indicated that there was no statistically significant correlation between these parameters in either experiment.

Large variations in salivary protein concentrations were noted within groups and between groups (Table I). No consistent relationship was noted to exist with strain, sex, or animal age. These salivary protein values probably are higher than the actual protein content due to the relative nonspecificity of this chemical determination. This likelihood is suspected because of the development of a small amount of blue color when the protein method was applied to the trichloracetic acid filtrate of saliva after protein precipitation.

The above data on salivary protease activity contrast sharply with the data published for the Hunt-Hoppert caries-resistant strains by Willett, Rosen, Stafseth, Hunt, and Hoppert.[1] Under the laboratory circumstances at Michigan State University, the protease activity in whole stimulated saliva from rats of the caries-susceptible strain that were 90 days old or older was 2.1 to 3.2 times as high as that of saliva from the caries-resistant rats of comparable age. Salivary protease activity increased sharply with age in the caries-susceptible rats but much smaller changes were noted with age among the caries-resistant rats.

The salivary protease values for the Hunt-Hoppert caries-susceptible and caries-resistant rats in the third experiment are presented in Table I. While there was considerable variability in salivary protease values within the various groups, it was obvious in the first half (a) of the experiment that the Hunt-Hoppert caries-susceptible rats, maintained on diet 700 + 15 per cent Cellu flour throughout the entire reproductive cycle under our laboratory conditions, had much higher average salivary protease values than the Hunt-Hoppert caries-resistant rats under the same conditions. In the second half (b) of the third experiment, no offspring were obtained from the caries-susceptible breeders on

the HWC diet. However, the salivary protease values among the Hunt-Hoppert caries-resistant rats maintained on the HWC diet were as low as the subjects from the same strain on diet 700 + 15 per cent Cellu flour.

The difficulties of breeding the Hunt-Hoppert caries-susceptible strain have been consistent throughout the 6 generations that they have been maintained in our laboratory. In general, the breeding animals of this strain have tended to reproduce more readily on ration 700 + 15 per cent Cellu flour than on the HWC diet.

The average scores for the extent of carious lesions among the Hunt-Hoppert rats in the third experiment are shown in Table II. These scores were obtained at the time of sacrifice when the rats were 110 days old. Under our laboratory conditions, rats of the Hunt-Hoppert caries-resistant strain have been appreciably less caries-resistant than the rats of the Harvard caries-resistant, and not a great deal more resistant than the rats of the Hunt-Hoppert caries-susceptible strain. The rats of the Hunt-Hoppert caries-susceptible strain have been in general about as caries-susceptible as rats of the Harvard caries-susceptible strain. In view of the increased caries-susceptibility of the Hunt-Hoppert caries-resistant strain, the ability to find approximately the same ratio of salivary protease activity between rats of the Hunt-Hoppert caries-susceptible and caries-resistant strains as had been reported by Willett and co-workers[1] at Michigan State University becomes even more striking.

DISCUSSION

In studies conducted at Michigan State University and in our laboratories, the salivary protease activity of mature rats from the Hunt-Hoppert caries-susceptible strain was consistently two to three times greater than the salivary protease activity of rats from the Hunt-Hoppert caries-resistant strain. In addition to repetition of the experiment with clearly corroborative results, the study in our laboratories indicated that the salivary protease values in the Hunt-Hoppert resistant rats on either the HWC diet or ration 700 + 15 per cent Cellu flour remained just as low in the presence of a significant incidence of carious lesions as the salivary protease values for caries-free rats of the same strain maintained under the conditions in the laboratories at Michigan State.

In contrast, no relationship exists between salivary protease activity and dental caries experience among representatives of the Harvard caries-resistant and caries-susceptible strains. The average salivary protease activities for the 2 Harvard strains were close to the average values for the Hunt-Hoppert caries-resistant strain. In general, the variability in salivary protease values for both Harvard strains was greater than for either Hunt-Hoppert strain.

Two major differences, the one dietary, the other genetic, exist in the management of the Hunt-Hoppert strains in the Michigan State laboratories and the Harvard strains in our laboratories. The Hunt-Hoppert colonies have been maintained routinely through the strain development program on the Hoppert-Webber-Canniff diet whereas the Harvard colonies have been maintained on purified ration 700 + 15 per cent Cellu flour. In our laboratories, portions of

the Hunt-Hoppert strains have been maintained on the 2 dietary regimens for comparative purposes. Since the salivary protease values for the Hunt-Hoppert strains were comparable for experiments conducted in the 2 laboratories, and with the 2 diets, any dietary variation apparently can be considered to be irrelevant.

The apparent reason for the divergent results would seem to be a result of genetic differences between the strains. The Michigan State breeding program has been based on the continued selection of breeders on the basis of brother-sister matings with departure from this program only when problems of virility were encountered. In contrast, the breeding animals in the Harvard strains have been selected in each generation on the basis of the phenotypic expression of tooth decay as determined by in vivo oral examinations at appropriate ages. Matings were made on the basis of the selection of a male and female with the closest available similarity in dental caries incidence with an attempt to have brother-sister matings alternating from generation to generation with matings between fairly closely related lines. Other characteristics such as general health, hair coat, reproductivity, resistance to respiratory infections, freedom from mammary tumors and from salivary gland infections were recorded and given weight in the selection process as means to maintain high virility simultaneously with the desired qualifications with respect to dental disease. The outcome of the 2 independent breeding programs, accidental without question, has been the development of the Hunt-Hoppert caries-susceptible strain with a simultaneous characteristic of a high salivary protease activity; in contrast, the Hunt-Hoppert caries-resistant and the 2 Harvard strains have not had the associated trait tending toward high salivary protease activity.

With these facts in mind, the question arose as to whether rats from the Harvard caries-susceptible strain could be inbred for high protease activity and independently for very low protease activity. A preliminary experiment of this nature is being carried out at the present time with several Harvard caries-susceptible animals as breeders which had exhibited extremely high levels of protease activity. The attempt to establish a substrain with very low protease values is being made with a group of breeders that had lower than average salivary protease values. In addition, a more definitive experiment with dietary manipulation to prevent formation of carious lesions is underway in order to determine conclusively if any relation exists between protease activity and susceptibility to dental decay in the Harvard and Hunt-Hoppert strains.

SUMMARY

1. Under our laboratory conditions, as in the laboratories at Michigan State University, the rats of the Hunt-Hoppert caries-susceptible strain demonstrated strikingly higher protease activity levels in saliva than did the rats of the Hunt-Hoppert caries-resistant strain. This ratio was consistent when ration 700 + 15 per cent Cellu flour was fed instead of the Hoppert-Webber-Canniff diet, and also when the rats of the Hunt-Hoppert caries-resistant strain developed extensive carious lesions.

2. In contrast, rats of the Harvard caries-susceptible and caries-resistant strains exhibited low protease activity levels in the saliva with no significant difference between the caries-susceptible and caries-resistant animals.

3. In view of the above findings, it would appear that the tendency toward high salivary protease levels in the caries-susceptible Hunt-Hoppert animals is a genetically determined trait that has appeared simultaneously with the tendency toward high caries-susceptibility. On the same basis, it would also be suspected that salivary protease activity, per se, is not directly related to dental caries.

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PROCEDURES FOR COMPUTING THE MEAN AGE OF ERUPTION OF HUMAN TEETH

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SEVERAL different methods have been used for computing the mean ages of eruption of the teeth from cross-sectional population data. The earlier methods have been reviewed by other authors.[1, 2] Some of the methods, such as those used by Cattell[1] and Cohen,[3] are primarily graphical procedures which require no assumption about the underlying distribution of the ages of eruption. These procedures yield good descriptions of tooth eruption for the group being studied but they do not permit judging the significance of differences among tooth types or among populations. Other investigators have suggested that the ages of eruption of a tooth conform to the normal probability curve.[2, 4, 5] The procedures used by Boas[4] and Klein[2] and their co-workers yield estimates of the mean and standard deviation of the ages of eruption but neither of these authors discusses technics for making statistical comparisons between populations. Clements, Davies-Thomas, and Pickett[5] applied a more complicated statistical procedure which includes provisions for computing the confidence limits of the mean. This latter procedure, then, provides for estimating the significance of differences in mean ages of eruption, but the computations are so arduous that it may not be suitable for routine use by dental investigators. Both Klein[2] and Clements[5] found that the distribution of the ages of eruption was satisfactorily described by the normal probability curve.

Another team of investigators, Kihlberg and Koski,[6] suggested the use of the logarithms of the ages of eruption, with conception as the zero point of age. These investigators found that these logarithms distribute normally and that the logarithmic standard deviations of the different tooth types have similar magnitudes. This suggestion that the logarithms of the ages of eruption distribute normally differs from the findings of other investigators.[2, 5]

The present report introduces a simpler procedure for computing eruption statistics and compares the results with those obtained by more complex procedures. It also attempts to test, for a selected tooth type with a relatively uncomplicated eruption pattern, the assumptions that (a) the ages of eruption are normally distributed, and (b) the logarithms of the ages of eruption are normally distributed.

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THE DATA

The basic data for this report are taken from the prefluoridation examinations of Russell's longitudinal study,[7] in which a tooth was considered to be erupted if any portion of the crown had penetrated the oral mucosa. For the purpose of this report, the mandibular canine tooth was selected as the typal tooth since it is rarely congenitally missing and its eruption is presumably unaffected by early loss of deciduous teeth from caries. However, children with a positive diagnosis of congenitally missing teeth, of any type, were excluded to reduce the probability of including a child with congenitally missing mandibular canines. No estimation of the number of impacted mandibular canines was attempted.

Any reported age which did not conform to the child's grade level was checked against subsequent examination records and/or against information from siblings. Those children whose ages did not conform and could not be confirmed were excluded from the study. The basic data were then tabulated, by month of age, and sex, as the number of persons with zero, one, or two teeth in eruption. The age range, corrected to the midpoint of the step-interval, was 5.04 to 16.96 years for the 1,801 boys and 5.04 to 16.62 for the 1,776 girls.

The histories of previous residence of the children were examined during the early stages of the study of the eruptive process. The eruption patterns of those children who had not resided continuously in the study area did not differ importantly from the eruption patterns of those children who had resided continuously in the area. Therefore, residence histories were not used to exclude children from this eruption study.

METHODS AND PROCEDURES

The simplest procedure used was Cornfield and Mantel's[8] adaptation of Kärber's method,* hereafter designated simply as Kärber's method. Its use is appropriate whenever the data effectively cover the range from zero to 100 per cent response as the present data do. Although this method has been used primarily for estimating the median and standard deviation of the normal distribution, it is a valid procedure for estimating the mean and standard deviation of any distribution.

The primary advantage of Kärber's method lies in its ease of computation. The computations are simple and need be done only once for any given set of data. This contrasts with the complex repetitive calculations required by the methods of maximum likelihood which are discussed later.

The computation procedures required by Kärber's method are especially simplified in the case of equally spaced data. In terms of arithmetic age (i.e., ordinary age from birth), the present data are effectively equally spaced, the interval of spacing being 1 month. The application of Kärber's method to a set

*A method similar to Kärber's method was used earlier by Röse[9] for computing the mean age of eruption of teeth. The Cornfield-Mantel adaptation can also be considered an extension of the method used by Röse, with simplified computing procedures.

TABLE I

KÄRBER'S METHOD AS USED FOR ESTIMATION OF ERUPTION CONSTANTS, MANDIBULAR CANINES, FEMALES, PRINCE GEORGES AND MONTGOMERY COUNTIES, MARYLAND, 1952

AGE IN YEARS AND MONTHS	NUMBER OF TEETH n	NUMBER OF TEETH ERUPTED	PROPORTION ERUPTED p	CUMULATED p	AGE IN YEARS AND MONTHS	NUMBER OF TEETH n	NUMBER OF TEETH ERUPTED	PROPORTION ERUPTED p	CUMULATED p
6-9	12	0	—	—	9-9	34	19	.559	6.121
6-10	14	2	.143	.143	9-10	18	14	.778	6.899
6-11	30	0	—	.143	9-11	24	18	.750	7.649
7-0	34	0	—	.143	10 0	44	33	.750	8.399
7-1	34	0	—	.143	10-1	24	16	.667	9.066
7-2	20	0	—	.143	10-2	36	27	.750	9.816
7-3	40	1	.025	.168	10-3	38	32	.843	10.659
7-4	26	0	—	.168	10-4	46	38	.826	11.485
7-5	30	1	.033	.201	10-5	36	31	.861	12.346
7-6	32	0	—	.201	10-6	22	16	.727	13.073
7-7	26	3	.115	.316	10-7	26	22	.846	13.919
7-8	24	0	—	.316	10-8	40	33	.825	14.744
7-9	14	0	—	.316	10-9	32	23	.719	15.463
7-10	24	1	.042	.358	10-10	24	23	.958	16.421
7-11	32	1	.031	.389	10-11	28	25	.893	17.314
8-0	34	4	.118	.507	11-0	24	23	.958	18.272
8-1	24	0	—	.507	11-1	20	18	.900	19.172
8-2	40	1	.025	.532	11-2	30	29	.967	20.139
8-3	42	3	.071	.603	11-3	32	32	1.000	21.139
8-4	32	3	.094	.697	11-4	24	24	1.000	22.139
8-5	46	6	.130	.827	11-5	22	20	.909	23.048
8-6	30	5	.167	.994	11-6	30	30	1.000	24.048
8-7	28	5	.179	1.173	11-7	20	19	.950	24.998
8-8	26	4	.154	1.327	11-8	36	35	.972	25.970
8-9	18	3	.167	1.494	11-9	22	21	.955	26.925
8-10	36	5	.139	1.633	11-10	22	22	1.000	27.925
8-11	26	7	.269	1.902	11-11	40	38	.950	28.875
9-0	20	6	.300	2.202	12-0	30	30	1.000	29.875
9-1	40	14	.350	2.552	12-1	34	34	1.000	30.875
9-2	42	17	.405	2.957	12-2	44	42	.955	31.830
9-3	52	12	.231	3.188	12-3	32	31	.969	32.799
9-4	22	7	.318	3.506	12-4	40	40	1.000	33.799
9-5	34	16	.471	3.977	12-5	42	42	1.000	34.799
9-6	28	11	.393	4.370	12-6	28	28	1.000	35.799
9-7	26	18	.692	5.062	12-7	36	35	.972	36.771
9-8	34	17	.500	5.562	12-8	44	44	1.000	$37.771 = S_1$
								Sum of the cumulated p's =	$809.062 = S_2$

Arithmetic Solution

$X_s = 12 \times 12 + 8 + 0.5 = 152.5$ months

$d = 1/12$ years

$M = d(X_s - S_1 + 1/2) = 9.6024$ years

$$S.D. = \sqrt{\frac{2S_2 - S_1(S_1 + 1) - 1/12}{d^2}}$$

S.D. is the standard deviation

S.D. = 1.0329

B = 1/S.D. = 0.9681

A = 5 − MB = −4.296

Probit equation: Y = −4.30 + 0.968X where X = age in years

$$S.E.(M) = d\sqrt{\Sigma\left[\frac{p(1-p)}{n-1}\right]} = 0.0407$$

Re-interpretation of the Arithmetic Solution (i.e., Logarithmic Solution)

$$C^2 = \frac{(S.D.)^2}{M^2} = 0.0115700$$

$(s.d.)^2 = (\log e) \log(1 + C^2)$

$(s.d.)^2 = 0.43429448 \times 0.00499593 = 0.00216970$

s.d. = 0.046580

b = 1/s.d. = 21.468

m = log M − 1/2 [log(1 + C^2)]

m = 0.97988

antilog(m) = ED_{50} = 9.547 years

a = 5 − mb = −16.036

Probit equation: Y = −16.04 + 21.47x where x = logarithm of the age in years

Note.—Data prior to age 6-9 and subsequent to age 12-8 are not shown. The eruption rates for the periods omitted were 0 and 100 per cent, respectively, and their omission does not affect the results of the calculations shown.

of data is illustrated in Table I. In the example, the mean age of eruption of mandibular canines in the girls is M = 9.60 years and the standard deviation is S.D. = 1.03 years. If the arithmetic age of eruption of teeth is assumed to have a normal distribution, then the probit,* Y, of the per cent erupted at any age X can be estimated as $Y = A + BX$, where $B = 1/S.D.$ and $A = 5 - MB$. In the example, this becomes $Y = -4.30 + 0.968X$.

If, however, age of eruption is not assumed to have an arithmetic normal distribution, but is assumed rather to have a log normal distribution (i.e., the logarithm of the age of eruption is normally distributed†), the calculation would have to be modified. An equal arithmetic interval of 1 month in the ages results in an unequal logarithmic interval, which in turn complicates the application of Kärber's method. A re-interpretation of the results already obtained avoids this difficulty.‡ This re-interpretation, illustrated in the lower right section of Table I, yielded a mean logarithm for the age of eruption, m = 0.9799 (the arithmetic median age of eruption is then 9.55 years in comparison with the arithmetic mean of 9.60 years) and the standard deviation for the logarithms of age of eruption, s.d. = 0.0466. The probit, Y, of the per cent erupted at any age x is then $Y = a + bx$, where $b = 1/s.d.$, $a = 5 - mb$, and $x = \log X$. In the present case this becomes $Y = -16.04 + 21.47x$.

Probit procedures were also used. These procedures, which assume an underlying normal distribution, require an initial entering approximation of the mean and standard deviation which can be derived from Kärber's method or from a tentative graphic solution. From this initial approximation, probit values are assigned to each point. These probit values and the observed eruption data are used to compute a better estimate of the mean and standard deviation. The process is continued, using each solution as the entering approximation for the next cycle of computations, until there is no important change in the values of the mean and standard deviation.

Probit procedures employing the method of maximum likelihood can be used assuming either an arithmetic normal or log normal distribution of the age of eruption. For purposes of fitting the log normal distribution there would be a

*A probit is a function of the distance, in standard deviations, of any point from the mean. The probit, Y, for any point X is given by the equation: $Y = 5 + \frac{1}{S.D.}(X - M)$, where S.D. is the standard deviation and M is the mean.

†In this report we have used the logarithm of the age from birth rather than the logarithm of age from conception. A preliminary study[10] indicated that both transformations yield essentially the same constants.

‡It can be shown that if a variable x (in this case the logarithm of the age of eruption) is normally distributed with mean m and standard deviation s.d. then the distribution of its antilog $X = 10^x$ (in this case arithmetic age) though non-normal will have mean M, standard deviation S.D. and coefficient of variation $C = S.D./M$, such that

$$m = \log M - \frac{1}{2}\log(1 + C^2)$$

and

$$(s.d.)^2 = (\log e)\log(1 + C^2).$$

Conversely

$$M = \text{antilog}\left[m + \frac{(s.d.)^2}{2 \log e}\right]$$

and

$$S.D. = M\sqrt{\text{antilog}\left[\frac{(s.d.)^2}{\log e}\right] - 1}.$$

separate log group for each month of age, and further, these would be unequally spaced. To simplify the calculations and to reduce the number of groups that would have to be handled, the ages were grouped into equally spaced log intervals. The same initial entering approximation was not used for each instance but, in all cases, the calculations were concluded when the change in the slope, b, was 0.04 or less. (The reported slope should then be within 0.1 per cent of the value obtained by continuing until the solutions converge and the standard deviation within the limits of the rounding of the reported arithmetic results.)

Analyses were made by two maximum likelihood methods and by Kärber's method with the data grouped into equal log intervals. First, the logarithms of age were grouped in intervals of 0.02 and the data analyzed by the Bliss-Fisher method of maximum likelihood, as described by Finney.[11] For this analysis some of the terminal age groups with zero and 100 per cent response were omitted as these would have added to the burden of computation while affecting the results only slightly. Next, the logarithms of age were grouped in intervals of 0.01 and the data were fitted by the Cornfield-Mantel method of maximum likelihood. For this analysis only data falling outside the zero to 10 probit range were omitted. In theory, both the Cornfield-Mantel and Bliss-Fisher methods converge for a given set of data to the same result. In practice, the results differ because of the rounding effects, these effects being somewhat more serious for the Bliss-Fisher method. The computations required by the Cornfield-Mantel method are a little simpler and, in addition, theoretically, converge more rapidly to the final result. Finally, Kärber's method was applied with the logarithms of age grouped in 0.01 intervals. For each of these solutions in which log age was used, the results were re-interpreted to yield arithmetic values, using the appropriate relationships shown in the footnote (‡) on page 941.

An underlying arithmetic normal distribution of the age of eruption was then assumed and the data were fitted by the Cornfield-Mantel method. To simplify the calculations with the Cornfield-Mantel method, the ages were regrouped into 3-month intervals.

The data were tested, by the Chi-square test, for goodness of fit to each of the calculated probit equations. In computing Chi-square values, those groups falling outside the zero to 10 probit range were omitted, and the other terminal groups were combined so that the minimum expected frequency in a group, erupted or unerupted, would be 8 teeth.

RESULTS AND INTERPRETATION

Table II shows the results obtained by applying the methods indicated to data on the eruption of mandibular canines in boys. For each method this table shows the computed mean age of eruption, standard deviation, and probit slope, together with the standard errors of the mean and the probit slope. The standard errors were computed on the basis of the number of teeth rather than on the basis of the number of children. By so doing it was essentially assumed that the eruption ages for the left and right mandibular canines in an individual are

independent. This assumption is probably not completely true but, at worst, if the eruption ages were perfectly correlated, the standard errors would be increased by a factor of $\sqrt{2}$, or about 40 per cent. Standard error procedures have not been developed for the constants which were converted from one scale to the other, but these standard errors should be close to the corresponding values shown.

TABLE II

COMPARISON OF TOOTH ERUPTION CONSTANTS, COMPUTED BY DIFFERENT PROCEDURES, MANDIBULAR CANINES, MALES

A. Arithmetic Solution Assuming an Arithmetic Normal Distribution of Ages of Eruption

LINE	PROCEDURE	MEAN AGE (M) IN YEARS ± STANDARD ERROR	STANDARD DEVIATION S.D. IN YEARS	B (1/S.D.) ± STANDARD ERROR
1	Kärber's* 1-month intervals	10.54 ± .05	1.09	.921 ± .031
2	Cornfield-Mantel 3-month intervals	10.58 ± .04	1.08	.922 ± .031

B. Arithmetic Solution Assuming a Logarithmic Normal Distribution of Ages of Eruption

LINE	PROCEDURE	MEAN AGE (M) IN YEARS	STANDARD DEVIATION (S.D.) IN YEARS	B (1/S.D.)
3	Kärber's* 0.01 log intervals	10.53	1.08	.925
4	Finney 0.02 log intervals terminal 0 and 100% points censored	10.55	1.08	.927
5	Cornfield-Mantel 0.01 log intervals	10.55	1.07	.934

C. Logarithmic Solution Assuming a Logarithmic Normal Distribution of Ages of Eruption

LINE	PROCEDURE	MEAN LOG AGE (m) ± STANDARD ERROR	ANTILOG m (MEDIAN) ± STANDARD ERROR, YR.	STANDARD DEVIATION (s.d.) LOG YEARS	b (1/s.d.) ± STANDARD ERROR
6	Kärber's 1-month intervals	1.0208	10.49	.0446	22.42
7	Kärber's* 0.01 log intervals	1.0203 ± .0018	10.48 ± .04	.0445	22.49 ± .78
8	Finney 0.02 log intervals terminal 0 and 100% points censored	1.0211 ± .0017	10.50 ± .04	.0443	22.57 ± .73
9	Cornfield-Mantel 0.01 log intervals	1.0211 ± .0017	10.50 ± .04	.0440	22.75 ± .73

The estimates of Lines 3, 4, 5 and 6 are derived from those of Lines 7, 8, 9, and 1, respectively. The relationships between the arithmetic moments and the antilogarithmic moments, shown in footnote ‡ of the text, were employed. Procedures for obtaining standard errors of such derived estimates have not been developed. No derived estimates corresponding to Line 2 are shown; these would involve certain theoretical difficulties.

*For Lines 1 and 7 the distribution is assumed to be arithmetic normal and logarithmic normal, respectively, only for purposes of calculating standard errors of the estimates. Use of Kärber's method does not require such assumptions for estimation of the mean and standard deviation only. Cornfield and Mantel[5] provide formulas for the standard errors of the mean and of the standard deviation as estimated by Kärber's method. The standard error formulas appear both in terms of the underlying percentages and, for purposes of calculation, in terms of the observed percentages. For comparability with other standard errors in this table we have employed the formulas expressed in terms of the underlying percentages. Calculated values of the underlying percentages were obtained from the Kärber's method estimates on the assumption of normality. In Table I, the standard error formula shown is in terms of the observed percentages.

The estimates of the arithmetic mean age of eruption (M) in Table II show good agreement among the different methods. The Cornfield-Mantel solution based on the assumption of a normal distribution of ages (Line 2) is 10.58 years as compared with 10.54 years for Kärber's method (Line 1). This difference of about 15 days does not exceed the standard error of either estimate of the mean. The results from the use of the maximum likelihood methods with the assumption that the logarithms of age are normally distributed (Lines 4 and 5) differ from the Kärber's solution (Line 3) by about 7 days. None of these variations appear to be important.

There is also good agreement among the estimates of the arithmetic standard deviation (Table II). The estimates computed from arithmetic data (Lines 1 and 2) differ by about 4 days, as do those based on log data (Lines 3, 4, and 5). The largest difference among the computed values of this constant is 7 days, between Kärber's method, 1-month intervals (Line 1), and the Cornfield-Mantel solution, 0.01 log intervals (Line 5).

All procedures yielded similar estimates for the ages at which 5 per cent and 95 per cent of these teeth were erupted. The extreme values for the age of eruption of 5 per cent of these teeth were 8.36 years from Kärber's method, arithmetic data, and 8.42 years from the Cornfield-Mantel method, arithmetic data. For the age of eruption of 95 per cent of these teeth the extreme values were 12.69 and 12.74 years. The estimates for each point have a range of less than 1 month.

The computed values for the arithmetic median age of eruption (Lines 6, 7, 8, and 9 in Table II) fall within one standard error of the median. The extreme values are 10.48 and 10.50, a range of about 7 days.

The data in Table II suggest that there is little choice between the arithmetic and logarithmic scale for estimating the age of eruption of teeth. The results from the Chi-square goodness of fit tests tend to confirm this suggestion. None of the procedures yielded a significant Chi-square value, the probability values for the Chi-squares ranging from 0.11 to 0.75. No particular method showed any superiority in this respect, and the assumption that the ages of eruption were log normally distributed was apparently not superior to the assumption of arithmetic normality. Neither of the assumptions would appear to be unreasonable.

The coefficients of variation, S.D./M, also indicate that the distribution of the ages of eruption can be described as either arithmetic or log normal. These coefficients were 0.10 for males and 0.11 for females. When the coefficient of variation of a log normal distribution is small, the distribution will be approximately symmetric and will somewhat resemble an arithmetic normal distribution. Conversely, arithmetic normal distributions with small coefficients of variation differ only slightly from the log normal. In the present instance, both assumptions adequately describe the data, reflecting the relatively small coefficient of variation observed in the ages of eruption. The choice of the assumption to use perhaps should be governed by the interest of the investigator.

DISCUSSION

Although the mandibular canine was selected because relatively few complications could be expected in its eruption pattern, the limitations of the examination and confirmation procedures make it likely that there was some residual error. There were, in the tails of the eruption curves, some very unusual data which could not be established as abnormal. Use of radiographs and a more detailed case history might have established that some of these apparently unusual occurrences were procedural errors or were actual abnormalities such as impacted or congenitally missing teeth. As the statistical procedures are very sensitive to data in the tails of the eruption curve, it is possible that exclusion of a few such cases would have resulted in a clearer indication that either the assumption of an arithmetic normal distribution or an assumption of a log normal distribution of the ages of eruption is superior. In any event, it appears that the accuracy of the data is more important than the assumption selected.

Since the use of Kärber's method is not dependent on the existence of normality, either arithmetic or logarithmic, in the ages of eruption, any uncertainties as to the existence of such normality would lend support to its employment in preference to the maximum likelihood methods.

Kärber's method, as presented in this report, should, of course, only be used when the observations adequately cover the entire range from zero to 100 per cent erupted. If this method is applied to incomplete data, the resulting estimate of the standard deviation will be too small. Lack of data at the lower ages, below the mean, will result in an overestimation of the mean; an underestimation will result if data are incomplete at the older ages. With such data it might be preferable to assume a normal distribution of ages of eruption and apply a maximum likelihood procedure. In most such instances, however, a graphic approach would probably yield a useful solution with considerably less effort.

When complete data are available, Kärber's method appears to be the method of choice for analysis of data on tooth eruption. Its application is simpler than the application of maximum likelihood methods; it requires no assumption about the underlying distribution; and it yields estimates that are not importantly different from estimates based on assumptions of either normal or log normal distributions of the ages of eruption. It is, therefore, recommended for use by dental investigators.

In some situations, the problem of congenitally missing teeth may be important enough to require adjustment of the computation procedures. If the proportion of congenitally missing teeth is known, or can be safely assumed, it is possible to adjust the proportion of erupted teeth and apply Kärber's method. The adjustment is given by the formula: $p = p'/(1-c)$ where p' is the observed eruption rate, c is the proportion of congenitally missing teeth, and p is the adjusted eruption rate. The formula for the standard error M then becomes:

$$\text{S.E.}(M) = \frac{d}{1-c}\sqrt{\Sigma\left(\frac{p'(1-p')}{n-1}\right)}$$

If the value of c is not known and cannot be safely assumed it may be necessary to apply a maximum likelihood procedure. Finney[11] shows how a maximum likelihood procedure can be used to estimate the natural response rate, as well as other parameters, from the data. These procedures can be adapted to the case of the congenital nonresponse rate.*

SUMMARY AND CONCLUSIONS

Data from a longitudinal study were used to investigate the distribution of the age of eruption of permanent teeth and to select an appropriate method for computing the statistical constants which describe this distribution. The mandibular permanent canine was selected for the study, because the data adequately covered the complete range of the ages of eruption and the eruption of this tooth is subject to few complications. Those children whose reported ages were demonstrably incorrect and those with a diagnosis of congenitally missing teeth were excluded prior to analysis.

The data were analyzed by maximum likelihood procedures, which are relatively complex repetitive procedures, and by Kärber's method, which is relatively simple. The application of Kärber's method to a set of data was illustrated. Kärber's method was applied to data on the eruption of the mandibular canines in males, and the results were compared with those obtained from use of maximum likelihood procedures.

The assumptions of logarithmic and of arithmetic normality in the distribution of the ages of eruption were examined. Both assumptions were apparently reasonable, and there was no clear indication in the data that either form of normality was the more appropriate.

By Kärber's method, applied to arithmetic data, the mean age of eruption of the mandibular canines in boys was found to be 10.54 years, with a standard deviation of 1.09 years. These values were neither significantly nor importantly different from any of the estimates obtained from use of the maximum likelihood methods. Inasmuch as Kärber's method yields essentially the same results, is easiest to apply, and requires no assumption concerning the type of distribution it was recommended for use, in preference to the maximum likelihood methods, whenever eruption data adequately cover the range from zero to 100 per cent erupted.

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*Finney[11] employs Abbott's formula, $P = (P'-C)/(1-C)$, to get adjusted response proportions. In Abbott's formula P′ is the observed response rate (mortality), C is the natural response rate (natural mortality), and P is the adjusted response. For the formula shown in the text, p′ has been defined as the observed response (eruption), c as the natural nonresponse rate (congenitally missing teeth), and p as the adjusted response. In both instances, the numerator is the proportion which has responded to the given stimulus and the denominator the proportion which will eventually respond if the stimulus is increased sufficiently. In principle, the case of natural response and congenital nonresponse are the same, but the procedures for solving problems of natural response must be modified to fit the special case of congenital nonresponse.

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RESEARCH ANNOTATIONS*

NEW MATERIAL FOR SOMATO-PROSTHESIS

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WHILE many excellent materials are available for the construction of prosthetic appliances for the treatment of surgical and other deformities of the intraoral region, no wholly satisfactory material is at present available for the construction of appliances designed to replace extraoral structures. This note describes a new material developed in this institution which should help to remedy this deficiency.

Appleman (*J. Pros. Dent.* 2: 401, 1952; *Oral Surg., Oral Med., & Oral Path.* 6: 1386, 1953) used a polyvinyl chloride derivative (Elastomer No. 105, Formulation No. 15K, Elastomer Corp., Nutley, N. J.) as the base material for extraoral appliances. Coloring was achieved through the use of oil-soluble dyes incorporated into the body of the material while it was still liquid and also onto the finished surface after polymerization. Polymerization was accomplished in a plaster or stone mold with dry heat at 100° C. for 6 hours. While his results were encouraging and helped many patients to lead a relatively normal life again, difficulty was experienced in reproducing natural colorings accurately. Moreover, the appliances made with this material did not last well over long periods and had to be frequently remade. Thin edges of the material would lose their resiliency and crack; the color stability was poor over long periods of wear.

Because of the shortcomings of the polyvinyl chloride derivative, a search was made for a new material which would not have these disadvantages and which would also be easier to fabricate in the dental laboratory. It was found that a combination of a silicone rubber (Silastic R.T.V. 501; Dow Corning Corp., Midland, Mich.) and various methyl methacrylate powders used to tint regular acrylic denture base material, in conjunction with a self-curing acrylic liquid with the addition of hydroquinone, provided a base material which showed good quality of flow during processing, and reproduced tissue colorings very well. The proper depth of coloring of specific areas was achieved by first lining the mold with a thin layer of untinted base material. Then, colored acrylic powders were sprinkled over this in the required areas, and the bulk of the appliance was filled with untinted base material. Polymerization was accomplished in a stone mold at room temperature for 18 to 40 hours depending on the bulk of the prosthesis.

Much additional investigation in both the laboratory and the clinic is required before this material is perfected. However, the material possesses the following desirable qualities: (1) It is flexible. (2) It is dimensionally stable under all conditions so far tested. (3) It can be cemented to human skin satisfactorily with cosmetic adhesives. (4) It can be colored so that it accurately reproduces the appearances of human tissue. (5) Its texture closely simulates that of skin. (6) It can be molded with standard, currently used dental laboratory technics to any form required in the prosthetic reproduction of the human body.

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*Short notes with or without illustrations submitted to this section are reviewed and published promptly on one page of the JOURNAL in accordance with the previously announced editorial policy (*J. D. Res.* 37: 373, 1958).

STUDIES ON HUMAN MOUTH PROTOZOA

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A PRELIMINARY survey of the incidence of parasitic protozoa in the mouths of 50 patients revealed that 56 per cent harbored *Entamoeba gingivalis*, 62 per cent *Trichomonas tenax*, and 30 per cent both of these. One sample taken from the mouth of a woman 50 years of age who had traveled in Central and South America revealed, in addition to *E. gingivalis*, numerous leptomonad stages of *Leishmania braziliensis*. These results were determined from microscopic examination (wet smear) of scrapings from debris in carious cavities and in and around the gingival crevice within 2 hours after collection of the material. Leptomonads were also successfully cultured in Novy, MacNeal, and Nicolle's medium (Contrib. Med. Research [V. C. Vaughn], Ann Arbor, Mich., 1903, p. 549).

Further study of 300 additional patients revealed: 41 per cent harboring *E. gingivalis*, 22 per cent *T. tenax*, and 18 per cent both. Nineteen samples diagnosed as negative by original wet smear examination were found positive on culturing (6, *E. gingivalis;* 13, *T. tenax*) in Balamuth's egg-yolk fluid medium (*Am. J. Clin. Path.* 16: 380, 1946), Dobell and Laidlaw's solid egg slant medium (*Parasitology* 18: 283, 1926) and a combination of these, overlaying solid egg slants with egg yolk fluid. Cultures showed better growth when bacteria were inhibited by the addition of 200-300 units of penicillin G and 1,000-1,200 units of streptomycin per milliliter.

While both these protozoans were found not infrequently in mouths rated as "clean and healthy" (226 patients—*E. gingivalis* 31 per cent, *T. tenax* 11.5 per cent, both 8.0 per cent), *E. gingivalis* was demonstrated invariably and *T. tenax* frequently in cases of pyorrhea (12 patients—*E. gingivalis* 100 per cent, *T. tenax*, 58.3 per cent, both 58.3 per cent). A high incidence also occurred in patients showing early progressive stages of periodontosis (62 patients—*E. gingivalis* 66 per cent, *T. tenax* 53.2 per cent, both 46.7 per cent). Wet smears of samples from diseased mouths revealed as many as 5 *E. gingivalis* and 25 *T. tenax* per high-power field (650×) in contrast to only an occasional organism in cases of infection in "clean and healthy" mouths.

The average number of carious lesions per individual was 1.99 per cent for the infected patients and 1.63 per cent for patients who were not harboring the protozoa.

In general, the incidence of both *E. gingivalis* and *T. tenax* increased with age of patients. Findings by age groups were (1) 11 children 6-12 years—no protozoa; (2) 76 teenagers 13-19 years—*E. gingivalis* 21.1 per cent, *T. tenax* 3.9 per cent; (3) 89 patients 20-30 years—*E. gingivalis* 44.9 per cent, *T. tenax* 22.5 per cent; (4) 40 patients 31-40 years—*E. gingivalis* 55 per cent, *T. tenax* 32.5 per cent; (5) 56 patients 41-50 years—*E. gingivalis* 50 per cent, *T. tenax* 37.5 per cent; (6) 23 patients 51-60 years—*E. gingivalis* 60.9 per cent, *T. tenax* 39.1 per cent; (7) 5 patients 61-80 years—*E. gingivalis* 60 per cent, *T. tenax* none.

Approximately equal numbers of males and females were examined (males 151, females 149) and each sex showed about an equal incidence of protozoa (males, *E. gingivalis* 39 per cent, *T. tenax* 21.8 per cent; females, *E. gingivalis* 42.9 per cent, *T. tenax* 22.1 per cent).

The degree of salivary flow (slight, average, extreme) was not correlated in any way with the presence or absence of protozoa.

Both *E. gingivalis* and *T. tenax* were found capable of cytolysis of epithelial cells, erythrocytes, and leukocytes, and *E. gingivalis* commonly ingests red blood cells and nuclear fragments of white blood cells. Each of these protozoa shows, in certain stages of its life cycle, structural characteristics and behavior patterns characteristic of the other. *T. tenax* was observed to throw out short, blunt pseudopod structures, to form comparatively thick and

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sluggish "proboscis-like" flagella and, finally, to transform into quiescent "cyst-like" bodies closely resembling rounded, dormant forms of *E. gingivalis*. The phenomenon of complete transformation of certain coprozoic amoebae into flagellate forms *(Tetramitus rostratus)* and back again into rhizopod form, with reversal of polarity, was observed by both Bunting (*Proc. Nat. Acad. Sc.* 8: 294, 1922) and Hollande (*Arch. Zool. Exper. et Gen.* 83: 1, 1942). Pascher (*Arch. Protistenk.* 38: 81, 1917) pointed out that some representatives at least of every order of flagellates form pseudopodia.

THE EXPERIMENTAL PRODUCTION OF CARCINOMA OF THE TONGUE IN MICE

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INTEREST in carcinogenic hydrocarbons as etiological agents in mouth cancer is increasing. The failure of such known carcinogens as methylcholanthrene and dibenzanthracene to produce malignant change in the oral epithelium to which they have been topically applied has led to speculation concerning the resistance of the oral mucosa to the action of those carcinogens tested. Either there is some agent on the surface of or within the oral epithelium which inhibits the carcinogenic action of the polycyclic hydrocarbons, or the carcinogens are not able to gain access to the tissue; that is, they do not have the necessary portal of entry to produce their effects. In an attempt to clarify the pathogenesis of experimental oral carcinogenesis, the following work was done.

Methods and Results.—Mice were anesthetized with Nembutal. A short bevel 27-gauge needle attached to a tuberculin syringe was inserted parallel to the mucosal surface of the dorsum of the tongue on one side of the mid-line starting at the tip and passing posteriorly for a distance of 2 or 3 mm. Approximately 0.01 ml. of an aqueous suspension containing 0.3 mg. of methylcholanthrene (Firminger, H. F., and Stewart, H. L.: *J. Natl. Cancer Inst.* 12: 49, 1951) was deposited in the submucosa, producing a bleb approximately 2 mm. in diameter which remained for at least 3 hours after the needle was withdrawn. Two hundred and fifteen mice were injected using the methylcholanthrene suspension. An additional 100 mice were injected using the suspending medium without the carcinogen.

Two mice were killed approximately every 3 days for 3 months and then every 7 to 10 days for 12 months. All remaining mice were sacrificed at the sixty-fourth week when the experiment was terminated. The tongues of all animals were removed and fixed in cold Rossman's solution. Serial sections were prepared, and alternate sections were stained by various methods for cytochemical study. The cytochemical pathogenesis will be discussed at a later date.

After the sixteenth week, animals started to show malignant change in the epithelium. Of the 18 remaining animals sacrificed in the sixty-fourth week of the experiment, all but 3 exhibited carcinoma. It is interesting to note that many animals in both the experimental and the control groups had tongue tumors similar to the granular cell myoblastoma in man. Details of these tumors will be reported at a later date.

Discussion and Conclusions.—Surface applications of known chemical carcinogens to the oral mucosa of experimental animals fail to produce malignant disease (Levy, B. M.: *J. A. D. A.* 54: 514, 1957). Since carcinoma can be induced by injecting the carcinogen beneath the oral mucosa, some support is given to the suggestion that a portal of entry is necessary for carcinogenesis (Lacassagne, A., and Latarjet, R.: *Cancer Res.* 6: 183, 1946).

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

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I. PRESIDENT'S INAUGURAL ADDRESS

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This is my seventeenth consecutive meeting of the I. A. D. R. I recognize there are many others here who have longer records and I think they will agree that the sessions have become increasingly stimulating. After these meetings, one always returns to his laboratory or practice full of new ideas or plans, some of which become productive. There is every hope that this and subsequent meetings will be more stimulating, because we are already witnessing the early returns of increased activity in research on a broad scale in dentistry. This was made possible by a decision of Congress in 1956 to give strong support to research in the Health Sciences. It was their hope to reduce the prevalence of disease by greater concentration on research. This action was the culmination of long efforts by many leading professional people and scientists to obtain more financial aid for personnel, equipment, and facilities. The decision was helped to a great degree by the current successes in medicine, such as the effectiveness of the poliomyelitis vaccines, made possible only by basic research. Congress also recognized the need for more information about mental health, oral health, aging, and diverse diseases.

Research in dentistry has been short of personnel and facilities, as well as funds, for many years, in spite of strong demands and a continuing struggle

for better support. The liberal support finally awarded dentistry is attributable to the co-operative efforts of many individuals and organizations.

On the other hand—and this is hard to believe—the availability of the funds *created* problems and shed light on many more.

> For example, there was the problem of whether the funds should be used to foster research in the more inactive areas of the country or whether they should be added to the incomes of those already active?
>
> More funds meant more personnel. Where could they be obtained? Very few workers were being trained because of insufficient compensation.
>
> If personnel were found, the increase meant the need for more equipment and more space. How could these be obtained? How could laboratories expand when all available space was already occupied?

Much credit is due the various divisions of the National Institutes of Health, especially the National Institute of Dental Research and National Dental Advisory Council, for solutions to these problems. As a result, many, if not all, dental research groups have benefited. Here are some of the ways encouragement and financial assistance are being offered to undergraduate dental students to gain research experience while in school. The fellowships and other positions now available to students make the annual salary of $1,800 paid in 1938 look pretty sad, but it seemed a lot to me! Physical facilities which have restricted many research programs are either being improved or new buildings constructed and planned with matching government funds. Our outlook for the immediate future is good.

Just as the liberal arts colleges must begin preparing for a bumper crop of applicants in the near future, we must make plans for disseminating the results of a bumper crop of research work. For this reason, I venture to discuss briefly our communications and our meetings.

The desire for seeking answers to the questions of nature is the main attraction which research holds for most. Some hope for nothing more than a better understanding of nature, whereas others seek answers for specific purposes—prevention of disease, improvement in treatment, discovery of a new drug, etc. All become preoccupied with their work together with the regular reporting of their findings at meetings, such as this one, and by writing papers for publication. In most cases, publication is in a scientific or professional journal, but occasionally the work is prepared for nonscientific or nonprofessional journals. As far as dentistry is concerned, many research reports are presented at meetings of the I. A. D. R. or in the JOURNAL of DENTAL RESEARCH. Reports on dental research are given at other scientific meetings and papers are published in other journals and, although I have no specific figures, I believe this practice is increasing mainly because of the broadening scope of our inquiries. However, with the increase in number of people interested in and supporting dental research, it is likely that neither

our own meetings or our own journal nor other meetings and journals will be able to accommodate the volume of work presented. We must seek ways to increase the number of papers we can publish and to allow more workers to participate in our meetings.

Publication could be improved in the following ways:

1. By increasing the number of pages in the JOURNAL.
2. By publishing monthly instead of bi-monthly.
3. By including in each monthly publication not only a number of standard manuscripts, but also a series of short reports such as are published in "The Proceedings of the Society for Experimental Biology and Medicine."
4. By publishing a JOURNAL supplement. An offset or some other process could be used to bring reports to readers' attention rapidly and before they are out of date.

All of this would help, but it cannot be done without more money than we now have for our JOURNAL. We really need a "sugar daddy," if we are to continue to improve the JOURNAL OF DENTAL RESEARCH and its scope.

In spite of some shortcomings, we do pretty well at informing one another and other scientists of the results of our research, but our communications with the public are grossly neglected. Most scientists and practitioners either find it difficult to write for the laity without creating inaccuracies in technical information, or resort to meaningless phrases for fear of being misquoted or misunderstood. An editorial in "Science" this year[1] was directed at the general lack of scientific writing for the public. Warren Weaver of the Rockefeller Foundation believes one ought to strive (a) to advance the knowledge of the uninformed without misinforming, and (b) to prevent the necessary minor inaccuracies from blocking a subsequent path to the truth. In other words, "give 'em the straight dope and no malarky." This twofold goal, he calls "communicative accuracy." It is well recognized that some workers have more facility at this than others. For example, there's a great difference between the reception of the husband who greets his wife with "My dear, when I look into your face, time stands still" and the one who says, "My dear, your face would stop a clock." We must encourage those who are good at "communicative accuracy," if we are to create an interest in the public for dental research. Otherwise, most people will continue to have no idea what is going on in dental research, or even that there is such a thing! I don't believe we will make further major gains in obtaining support for research until we bring more of our results to the attention of nonscientists. The responsibility for this rests on *each of us*. Our annual meetings present an excellent opportunity to let the world know what is going on in dental research. Certainly we have had good publicity, but I believe we could be more thorough and improve it. Besides publicizing our own work, the annual meeting also provides a great opportunity for requesting publicity reports from our divisions and sections

overseas. These could appear simultaneously in their own countries and in the United States, and this, I believe, would help our colleagues overseas join in our meeting.

One last word about communications. The National Science Fairs[2] and other science talent searches have become popular. High school students have devoted some attention to problems in dentistry. For example, the recent Westinghouse[3] talent search had an exhibit on dentistry which gained a place in the top 40! We ought to support such activities whenever called upon; perhaps, we ought to assert ourselves and offer a prize for ideas or displays or experiments in dental research.

During a dental examiners' meeting in Chicago last February, Reidar Sognnaes made a statement about the fact that dental research is a well-kept secret. Words to the same effect were offered 20 years ago in the inaugural address by Dr. Gies.[4] If we're ever going to do something about this, we'll have to show more aggressiveness and ingenuity.

The immediate past is noted for our efforts to stimulate interest in and study of the biologic sciences as necessary groundwork for clinical studies. This firm foundation serves as a stimulus for clinical research and is worthy of our support. We need the clinician and the scientist side by side. I refer you to a most interesting article on professional collaboration in an issue of "Science" last year by Vannevar Bush.[5] The part that appealed to me was the thought that, if the scientist is to understand the clinician and vice versa, each must experience some of the problems of the other. He suggested that the clinician visit the laboratory often and, if at all possible, take time off to sit in the laboratory and learn some of its technics and language. The scientist should visit the clinician in like manner. Are our basic science people drifting too far away from the clinicians? Are the clinicians finding it difficult to communicate with the scientist? We have offered a common meeting ground in past years and I hope we will encourage more participation by clinicians.

Lastly, there are too few conferences or symposia on specific subjects in dental research. Our meetings are too tightly programmed to permit get-togethers by special groups. We have many outstanding workers and authorities in our membership, but when do we get to hear them? If at all, it's a 10-minute paper, often with little time for discussion. I believe the time has come to consider at least a one-half day extension of our meeting time, or a 4-day meeting. Such a development, as well as allowing more time for presentation of work and discussion, would allow time for special groups to hold meetings on specific topics. For such meetings we could call on the best people both within and outside the I. A. D. R. The extension would also allow an opportunity to continue co-operative meetings with the American Association of Dental Schools. This liaison with the teachers and school administrators is important, for teaching and research, no less than clinical practice and research, must not be separated. In this regard, we are all looking forward to the program scheduled for Sunday evening.

In conclusion, there are many problems now, and there will be more ahead of us, but with your advice, guidance, and co-operation, we will find satisfactory solutions. Thank you for your kind attention and patience. I hope you'll all be with us again in San Francisco.

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1. Weaver, W.: Communicative Accuracy, Editorial, *Science* **127**: 1958.
2. Morrey, L. W.: Science Fairs: An Opportunity to Interest American Youth in Dental Science, Editorial, *J. A. D. A.* **56**: 402, 1958.
3. Westinghouse Talent Search Finalists, *Science Newsletter* **73**: 166, 1958.
4. Gies, W. J.: Inaugural Address I. A. D. R., *J. D. Res.* **18**: 235, 1939.
5. Bush, V.: Professional Collaboration, *Science* **125**: 49, 1957.

II. EXECUTIVE PROCEEDINGS

(Compiled by Dan Y. Burrill, Secretary-Treasurer)

Executive Session I. March 20, 1958. 8:00 P.M.

With a quorum present, the meeting was called to order by President Sognnaes. The President extended greetings to visitors from outside North America and read messages of greeting to the Association from the Detroit Dental Society and from Edward H. Hatton.

The minutes of the 1957 meeting, printed as *Executive Proceedings* in the Journal of Dental Research (36: 800, 1957), were approved.

Tellers were appointed to count the ballots for election of officers (G. W. Burnett, Washington, D. C.; S. Wah Leung, Pittsburgh; and Gordon Nikiforuk, Toronto).

President Sognnaes reviewed the status of research publications, the increasing numbers of papers submitted and likely to be submitted for publication, and the delay in publication resulting from the volume of material. He acknowledged the sources of support for the Journal of Dental Research, namely, member and nonmember subscribers to the Journal, commercial grantors providing block subscriptions, and the Supporting Associates whose grants provide extra pages in the Journal. It was announced that the William J. Gies Foundation for Dental Research had just made a grant of $3,900 for 1958, to be used in the publication of more papers in the 1958 volume of the Journal of Dental Research. It was predicted that by the end of 1958 there would be a period of only 6 months between submission of an acceptable paper and its publication. Since processing time is around 6 months, the Journal will then be on practically a current basis. It was thought that few other journals could do any better, or as well.

The President reported that, in accordance with the instructions of the members, expressed at the 1957 meeting, the Association had become affiliated with the American Institute of Biological Sciences. The functions of the A. I. B. S. were explained and William G. Shafer was appointed representative from this Association in connection with a forthcoming meeting of the A. I. B. S. in Indianapolis during the summer of 1958.

In a report on the status of our membership in the Scandinavian countries, it was noted that after the election of a very few additional members the group would be eligible for division status in the Association.

The President reported on the 12th Congress of the Fédération Dentaire Internationale, held in Rome, September, 1957, and our relations with the F. D. I. He also urged members to support this organization.

Secretary-Treasurer Burrill submitted a copy of the yearly audit of the books and accounts of the Association (see supplement, A). The audit was accepted. The Secretary-Treasurer reported that with the increase in dues effective for 1958, the Association would be in a sound financial condition. This change will not be reflected in the accounts for 1957. He also reported on the completion of incorporation of the Association (under the laws of the state of Illinois) as a corporation not for profit.

The retiring Editor, Hamilton B. G. Robinson, presented a formal report concerning the JOURNAL OF DENTAL RESEARCH (see supplement, B).

Dr. René Rochon, as local arrangements chairman, welcomed the Association to Detroit and expressed his thanks to the officers and members of the Association for their assistance in preparing for the meeting.

H. Trendley Dean reported for the committee on meeting places. The schedule thus far planned is as follows:

1959—San Francisco, Sheraton-Palace
1960—Chicago, Morrison Hotel
1961—Boston, Statler Hotel
1962—St. Louis
1963—Pittsburgh
1964—Undecided
1965—Toronto, Washington, D. C., or Indianapolis

It was suggested that the Association should consider June instead of March as a time for the annual meeting.

After a report by Ned B. Williams as President-elect and as chairman of the Program and Publicity committees, the meeting was adjourned.

COUNCIL MEETINGS

1. March 21, 1958. 8:30 A.M.

Twenty-three members were present: F. A. Arnold, Jr., B. G. Bibby, B. Y. Burrill, H. D. Coy, A. K. Fisher, J. Gibilisco, R. J. Gorlin, J. Haldi, T. J. Hill, R. L. Ireland, W. Lefkowitz, B. M. Levy, P. L. Munson, F. J. Orland, S. Pearlman, S. Ramfjord, H. B. G. Robinson, W. G. Shafer, R. F. Sognnaes, L. M. Sreebny, H. A. Swanson, J. F. Volker, and N. B. Williams. (F. Earle Lyman [N.I.H.] and J. Roy Blayney were present as invited guests.)

President Sognnaes and Editor Robinson reported on the status of the JOURNAL of DENTAL RESEARCH, the costs of operation, and the contract with the publisher. It was agreed that the arrangements with the publisher had been favorable to the Association considering the limited funds guaranteed by membership dues.

It was voted that the record express the satisfaction of the group with the way the JOURNAL had been conducted and with the present arrangements for its publication, but it was further voted that the officers continue to review these arrangements with possibilities of future improvement in mind.

F. A. Arnold, Jr., reported the decision of the National Institutes of Health to approve initial financial support for a journal of clinical dental research. J. Roy Blayney and Thomas J. Hill spoke concerning the proposed journal. The council was informed that the proposed journal was not intended to duplicate the work of the JOURNAL of DENTAL RESEARCH, but was to be a journal for the dental practitioners who are not primarily involved in research work. It was pointed out that the JOURNAL of DENTAL RESEARCH would then be in a better position to publish the increased numbers of papers that would be produced through the research grant program of the National Institutes of Health in so far as each research grant could provide for payment, in part, of the costs of publishing the results of work under a given grant.

F. J. Orland, as editor-elect, submitted a list of names constituting an Advisory Editorial Board to assist in consideration of papers for publication in the JOURNAL. Approval of the board as named was voted. (The names appear inside the front cover of the JOURNAL.)

The committee appointed in 1957 to study the annual program (J. A. Gibilisco, G. C. Paffenbarger, and J. H. Shaw, Chairman) submitted a written report which was modified in discussion and was adopted as modified. The report approved most of the practices

which have been followed by the program committees in the past. It was suggested that symposia and round table discussions should be included in the program if this could be done without conflict with or replacement of scientific sessions. An increase in the number of simultaneous scientific sessions as warranted by increased quantity of qualified abstracts was recommended. One month between the deadline date for receipt of abstracts and the printer's deadline was considered the minimum time necessary for the work of the program committee. This interval would be longer and the deadline consequently earlier if preprinted abstracts are to be made available. Abstracts of papers presented at the meeting should be published in the JOURNAL of DENTAL RESEARCH, either before or after the meeting. The report also offered suggestions for the conduct of the scientific sessions.

The subject of honorary memberships in the Association was discussed, the Council deciding that such could be conferred. A committee was appointed to define suitable procedures and to report at the second council meeting.

2. March 22, 1958. 8:30 A.M.

Twenty-four members were present: F. A. Arnold, Jr., D. Y. Burrill, H. D. Coy, H. T. Dean, J. Gibilisco, J. Haldi, J. F. Hall, T. J. Hill, R. L. Ireland, H. T. Knighton, W. Lefkowitz, B. M. Levy, P. L. Munson, F. J. Orland, S. Ramfjord, J. R. Ring, H. T. Knighton, H. B. G. Robinson, W. G. Shafer, R. F. Sognnaes, L. M. Sreebny, H. A. Swanson, J. F. Volker, and Ned B. Williams.

H. Trendley Dean reported on the action of the Edward H. Hatton Award committee and suggested that a committee of three be appointed to study the rules of the competition. Several questions had arisen about these rules, notably the requirement that every co-author of an entry should be a novice. It was pointed out that while many studies may be carried out under N. I. H. grants awarded to one man who is designated as principal investigator, it is not necessary for his name to be included among the authors listed on each paper resulting from such a grant.

The Secretary-Treasurer requested approval of a budget for his office, the same as that approved for the previous year with the addition of an allowance for the purchase of a typewriter. The Council approved his request and allowed an additional sum for secretarial or stenographic assistance.

J. Frank Hall, retiring business manager of the JOURNAL and member of the publication committee, reported on the business of the JOURNAL and submitted his final accounting of funds belonging to the JOURNAL (see supplement, C).

The Council formally recognized that the members of the I.A.D.R. in Great Britain have achieved Division status.

Nominations to regular membership in the Association were considered and 50 persons were nominated for election by the Association. After discussion of the qualifications for membership in the Association and whether to broaden the basis of membership, it was voted that the President appoint a committee to consider the matter and report at the 1959 meeting.

In consideration of plans for future meetings, the council voted on the question of preprinted abstracts, raising the registration fee, and making the deadline for abstracts earlier if preprinted abstracts were to be produced. Since the vote was indecisive, the officers were instructed to learn the opinion of the members on this matter.

The Council voted 12 to 3 (some not voting) that there be no Read-by-Title section of the program for the 1959 meeting.

Dues were set for 1959 at the same level as for 1958: $15.00 for members in the North American Division, including subscription to the JOURNAL, and $3.00 for members outside North America, with subscription to the JOURNAL optional.

EXECUTIVE SESSION II. March 22, 1958. 5:00 P.M.

With a quorum present, the session convened as a meeting of the North American Division. Ward Pigman was elected representative to the American Association for the

Advancement of Science and S. Wah Leung was elected representative to Section Nd of the A. A. A. S. The session adjourned and immediately reconvened as a general meeting of the I.A.D.R.

B. G. Bibby, as chairman of a committee appointed for the purpose, presented a resolution of appreciation for the services of H. B. G. Robinson as editor and J. Frank Hall as business manager of the JOURNAL. The resolution was adopted and appears in the supplement.

A resolution was adopted, thanking Dr. René Rochon, Dean, School of Dentistry, University of Detroit, and the members of his committee for their successful handling of the local arrangements for the 1958 meeting.

The President presented the budget approved by the council for the office of the Secretary-Treasurer. Approval was voted. (The budget appears in the supplement, D.)

Payment of all expenses of the meeting was expressly authorized.

The State Bank and Trust Co. of Evanston, Illinois, was approved as the repository of Association funds.

The Secretary-Treasurer read the names of members who had died during the past year. The assembled members stood silent in tribute to their memory.

Fifty new members were unanimously elected into the Association. (List appears in the supplement, E.) Among the newly elected members were a number from the Scandinavian countries. Their election gave the Scandinavian group enough members to qualify as a division of the Association. The PRESIDENT called for a vote of councillors present on approval of the Scandinavian division. All voted approval. The Association extends greetings and best wishes to the new Division and to the British Division already given Council sanction.

A petition for amendment of the constitution—to open membership in the Association to all persons interested in research without distinction on the basis of participation in research—was presented.

Dr. Harold Hillenbrand was nominated for election to honorary membership in the Association and was unanimously elected. A citation honoring him appears in the supplement (F).

Edgar E. Coolidge was nominated for election as honorary Vice-president of the Association. He was unanimously elected. A citation appears in the supplement (F).

S. Wah Leung reported for the tellers the results of the election of officers for 1958-1959, as follows:

President	Ned B. Williams
President-elect	H. B. G. Robinson
Vice-president	Holmes T. Knighton
Secretary-Treasurer	Dan Y. Burrill
Editor	Frank J. Orland
Publication Committee	John B. Macdonald
	David F. Mitchell
Councillors-at-large	Ralph L. Ireland
	Howard J. Merkeley

Edward H. Hatton was re-appointed Assistant Secretary-Treasurer. The newly elected officers, including Vice-president Coolidge, were installed.

After expressing his thanks to the officers and his confidence in further growth of the Association Reidar F. Sognnaes turned the meeting over to the newly elected President. President Williams called on the new editor, Frank J. Orland, who discussed his plans for the JOURNAL of DENTAL RESEARCH. (See supplement, G.)

The meeting adjourned sine die.

The annual banquet on Friday, March 21, 1958, was attended by 240 persons. Ned B. Williams, then President-elect, presented his inaugural address on "The I.A.D.R. and Communications" (see Proceedings, page 951). J. F. Volker presented a biographical sketch of the retiring Editor, H. B. G. Robinson.

George C. Paffenbarger was presented the fourth annual Wilmer Souder Award in recognition of his outstanding services in improvement of dental health through dental materials research.

The Edward H. Hatton Award was presented to J. O. Beck, Jr., of Indiana University for his paper "BW 1081 Mouse Salivary Gland Tumor Growth Characteristics—Host Responses." Honorable mention was received by D. L. Anderson and R. C. Burgess, both of the University of Toronto.

On Sunday evening March 23, 1958, a joint meeting of the I.A.D.R. and the American Association of Dental Schools was held. The theme of the meeting was "Interrelationship Between Research and Education in Modern Dentistry." The speakers were: Roy O. Greep, Dean of Harvard University School of Dental Medicine; Harry Lyons, Dean of the Medical College of Virginia, and Dr. C. J. Van Slyke, Associate Director of the National Institutes of Health. Roy G. Ellis, President of the A.A.D.S., presided, and Reidar F. Sognnaes, President of the I.A.D.R., moderated the discussion following the talks. The papers and discussion are being published in the May issue of the *Journal of Dental Education.*

III. SUPPLEMENT TO PROCEEDINGS

A. FINANCIAL STATEMENT FOR YEAR ENDED DECEMBER 31, 1957

Office of the Secretary-Treasurer—Chicago, Ill.*

Fund Balance, January 1, 1957			
On deposit at Lincoln Bank and Trust Company, Louisville, Ky.		$ 5,014.96	
On deposit at State Bank and Trust Company, Evanston, Ill.		230.07	
Petty Cash Account		38.11	
Total Fund Balance			$ 5,283.14
Receipts:			
Subscriptions		$ 4,388.36	
Dues		2,769.85	
Registrations Fees and Sale of Banquet tickets		3,219.25	10,377.46
Total Receipts			$15,660.60
Disbursements:			
Printing and Distributing JOURNAL (C. V. Mosby Co.)		$ 5,173.00	
Mailing Operations, Postage and Printing		856.55	
Gratuity to Secretary-Treasurer		500.00	
Annual Meeting Expenses:			
Meeting Facilities and Banquet	$1,593.75		
Printing Programs, Badges, etc.	509.38		
Novice Award	100.00		
Abstract—Preparations and Mailing	335.04		
Projection Service	361.00		
Other	22.00	$ 2,921.17	
American Association of Biological Sciences Affiliation Fee		100.00	
Cost of Incorporating		135.00	
Miscellaneous and Office Expenses		175.22	
Total Disbursements			$ 9,860.94
Fund Balance, December 31, 1957:			
On deposit at State Bank and Trust Company, Evanston, Ill.		$ 5,865.52	
Less, Due to Petty Cash		65.86	
Total Fund Balance			$ 5,799.66

At December 31, 1957, the association owns office equipment which cost $183.77. Not included in the above statement—1957 expenditures of $127.11 which were paid in 1958.

*Audit by Dale H. Taylor, C.P.A.

B. REPORT OF THE RETIRING EDITOR OF THE JOURNAL OF DENTAL RESEACH

At the termination of 22 years of stewardship, it is appropriate not only to report for this year but also to report for the intervening years since 1936 when the JOURNAL of DENTAL RESEARCH was first published by the International Association for Dental Research.

There had been no JOURNAL published in 1935 and, for that reason, there is a combined 1935 and 1936 issue which was published under the auspices of the first Publication Committee appointed by the I.A.D.R. That committee consisted of Harold Hodge, Maynard Hine, and myself.

When the new committee, who were all Rockefeller Fellows at Rochester, took over. there was no subscription list, there were no resources available, and the JOURNAL carried no advertising.

J. Frank Hall began to assist as Business Manager and Richard Manly, Marion McCrea and others began to help the new Publication Committee as fellow members of the Rochester group. Between 1936 and 1947, J. Frank Hall actively served as Business Manager and he and I published the JOURNAL together for the Association on a pay-as-you-go basis. As subscriptions were sold, or additional funds received, Frank Hall informed me of the currently available funds and the JOURNAL was printed to the extent of those available resources. In this way, the JOURNAL was never actually in the red but was never in a firm financial position.

In 1948, the JOURNAL of DENTAL RESEARCH was first published by The C. V. Mosby Company. This was on the basis of an agreement between the I.A.D.R. and The C. V. Mosby Company. Discussions were held between the Editor and Business Manager, representatives of The C. V. Mosby Company and, with the advice of William J. Gies, the present plan was set up. Dr. Claude Wood, then Vice-president of the Mosby firm, was instrumental in helping effect this agreement.

In 10 years, the total loss by The C. V. Mosby Company from publishing the JOURNAL has been $24,872. The only two years in which the company was in the black for the JOURNAL were 1956 when they showed a profit of $778 and 1957 when they showed a profit of $525. In 1957, the cost of producing the JOURNAL was approximately $42,000 for which about $30,000 was for printing and mailing.

The subscription list is not stable except for approximately 700 members of the I.A.D.R. who pay for their JOURNAL along with their dues, and approximately 3,300 student subscriptions paid for by a grant from the Colgate-Palmolive Company. In a typical year, approximately 500 nonmember subscribers cancel and, chiefly due to promotion of subscriptions by the publisher, about 500 new nonmember subscribers are obtained. Growth of the subscription list occurs chiefly through new memberships. As of December, 1957, there were 5,688 subscribers, the majority of whom were students. Only about 1,500 nonmembers and nonstudents are subscribers.

The number of papers in the hands of the Editor and Publisher has been reduced so that after publication of the April issue, now in press, only about 75 papers will remain unpublished. These probably will all be published in the volume for 1958. Thus, the time between receipt of papers and their publication has been reduced to about 6 or 7 months. Since processing papers and publishing needs require a minimum of about 6 months between receipt and appearance of any paper, the JOURNAL is practically *current* in publication. This has been made possible, largely through the sustaining supporters who each contribute $100 or more per year to the JOURNAL, and through the co-operation of the publishers. Plans are now contemplated which should permit an orderly growth in size of the JOURNAL, and the problem of delay in publication should not be as acute during the immediate future.

Many groups and individuals deserve special thanks for their co-operation but to single them out would be almost impossible. However, one individual deserves especial

recognition. If it had not been for the devotion and ability of our Business Manager, J. Frank Hall, the JOURNAL could never have survived those extremely critical years between 1936 and 1948. Even after that, his counsel and guidance aided greatly in keeping The C. V. Mosby Company interested in continuing to publish a JOURNAL that was not only nonprofit making but actually a financial liability. I believe that the JOURNAL is entering a new era of development—that its Editorship and custody is passing into capable hands and that it will continue not only to develop but also to expand its content and its influence.

To the new Editor, Frank J. Orland, I wish as enjoyable and fruitful an editorial career as has been my fortune during the past 22 years.

HAMILTON B. G. ROBINSON, D.D.S.

C. REPORT OF BUSINESS MANAGER FOR THE JOURNAL OF DENTAL RESEARCH

March 15, 1957, through March 15, 1958

Balance on hand, March 15, 1957		$ 530.88
Receipts:		
Sale back issues	$ 333.73	
Supporting Associates	4,500.00	
Colgate-Palmolive Co.	16,736.00	
Total Receipts	$21,569.73	$21,569.73
Grand Total		$22,100.61
Expenditures:		
Long distance phone calls	26.14	
Postage	45.00	
Dues payment for Editor	10.00	
Petty cash	30.00	
Secretarial expenses	500.00	
C. V. Mosby Co. for extra pages		
4/ 6/57	1,000.00	
8/21/57	1,000.00	
2/10/58	1,780.00	
C. V. Mosby Co. for Colgate-Palmolive grant	16,736.00	
	$21,127.14	$21,127.14
Balance on hand, March 15, 1958		$ 973.47

J. FRANK HALL, D.D.S.
BUSINESS MANAGER

D. BUDGET FOR 1958-59

Office of Secretary-Treasurer

Direct Expenses:	
Clerical	$ 250.00
Postage	150.00
Supplies	150.00
Printing	600.00
Travel and Misc.	50.00
Edward H. Hatton Award	100.00
Contingency Fund	200.00
Gratuity	500.00
Allowance for purchase of electric typewriter	450.00
Allowance for secretarial services	1,500.00
Total	$3,950.00

Note: The amounts in each of the categories of direct expense are approximate. Transfers from one category to another as necessary are approved.

E. NEW MEMBERS ELECTED MARCH 22, 1958

NORTH AMERICA—Manuel M. Album, *Philadelphia, Pa.;* B. R. Bhussry, *Washington, D. C.;* R. L. Bowen, *Washington, D. C.;* Vernon J. F. Brightman, *Chicago, Ill.;* Charles Justin Burstone, *Indianapolis, Ind.;* Robert Graig Caldwell, *Birmingham, Ala.;* Gerald T. Charbeneau, *Ann Arbor, Mich.;* C. Keith Claycomb, *Portland, Ore.;* Willard G. Clements, *Pittsburgh, Pa.;* John Cornell, *Philadelphia, Pa.;* Arthur H. Craven, *East Lansing Mich.;* Reuben Feltman, *Passaic, N. J.;* Donald Emil Flieder, *St. Louis, Mo.;* Alvin F. Gardner, *Washington, D. C.;* Edward N. Green, *Brooklyn, N. Y.;* Bertram S. Kraus, *Seattle, Wash.;* George A. Krikos, *Rochester, N. Y.;* William L. Kydd, *Seattle, Wash.;* Edward Patrick Lynch, *Omaha, Neb.;* John F. Mortell, *Ann Arbor, Mich.;* George E. Myers *Ann Arbor Mich.;* Richard L. Myerson, *Cambridge, Mass.;* Richard D. Norman, *Indianapolis, Ind.;* Harold T. Perry, Jr., *Chicago, Ill.;* Samuel Pruzansky, *Chicago, Ill.;* Giuliano Quintarelli, *Boston, Mass.;* Robert Rapp, *Toronto, Can.;* Ira Franklin Ross, *East Orange, N. J.;* Sam Rosen, *East Lansing, Mich.;* Nelson W. Rupp, *San Diego, Cal.;* Samuel M. Standish, *Indianapolis, Ind.;* Paul R. Weisenstein, *Columbus, Ohio;* Robert V. Winders, *Milwaukee, Wis.;* Julian B. Woelfel, *Kensington, Md.;* Doran D. Zinner, *Coral Gables, Fla.*

OUTSIDE NORTH AMERICA—Peter Barnard, *Australia;* Henry Joseph James Blackwood, *England;* Wolfgang Buttner, *Germany;* Juan Chaneles, *Argentina;* Richard G. Earnshaw, *England;* Takao Fusayama, *Japan;* Arne Reinhardt Hagen, *Norway;* Albert Keil, *Germany;* Bo Krasse, *Norway;* Kaare Langeland, *Norway;* Tormod Mörch, *Norway;* Henry W. Noble, *Scotland;* Kaare Reitan, *Norway;* Shosaburo Takuma, *Japan;* Svein Utheim Toverud, *Norway;* John Richard Trott, *England;* B. J. Wagg, *England.*

F. RESOLUTIONS AND CITATIONS

Resolved: That the I.A.D.R. record its appreciation to Dr. Hamilton B. G. Robinson, retiring Editor, and Dr. J. Frank Hall, retiring Business Manager, of the JOURNAL of DENTAL RESEARCH. Dr Robinson and Dr. Hall have carried the major responsibility for the publication of the Association Journal for more than twenty years, during which time they have, by virtue of ingenuity and devoted labor, restored the JOURNAL from its state of tenuous existence in 1936 to its present condition of strength and virility. To Dr. Robinson and Dr. Hall and various members of the Publication Committee who have helped them, all members of our Association and everyone interested in dental research will forever be indebted.

Citation: It is the distinct pleasure of the council of the I.A.D.R. to nominate Dr. Edgar E. Coolidge, long-standing active member of I.A.D.R., as Honorary Vice-president for the coming year. This is in recognition of and appreciation for his more than fifty years of service as an investigator, teacher, and practitioner of dental science and especially for his outstanding contribution to our knowledge of the etiology and treatment of diseases of the pulpal and periodontal tissues.

Citation: The International Association for Dental Research recognizes the contributions that Dr. Harold Hillenbrand, secretary of the American Dental Association, has made in mobilization of the dental and allied professions to increased activities in the field of dental research. In recognition of this, the International Association for Dental Research wishes to confer honorary membership in the Association with this citation. Dr. Harold Hillenbrand has displayed great vision in the strengthening of resources for the present growth of research basic to a health service and its relation to the prevention and treatment of oral disease. He has made an outstanding contribution, as an administrator, to the securing of greatly increased support for dental research and the training of additional qualified investigators, to the end that the oral health of the nation may be better served.

G. FUTURE PLANS FOR THE JOURNAL

During the past few days many of you have asked me about the future of the JOURNAL of DENTAL RESEARCH. I would like to present you with some of my thoughts on the topic at this early stage.

A year ago, the Editorship of the JOURNAL was one of the remotest of concerns to me. Since then, of course, I have given the matter considerable thought both before accepting the nomination as well as prior to my currently announced election. I feel moved to thank you for your confidence and to assure you that I will accept the office as a challenge and as a privilege to serve the Association.

In recent months, I have learned a great deal about the JOURNAL and its problems. I can thus appreciate the remarkable job Hamilton Burrows Greaves Robinson has done as Editor. Likewise, I am also appreciative of the commendable task J. Frank Hall has carried out over the years on the business end of the JOURNAL.

Now let's be concerned about the JOURNAL per se. You've heard about its financial "Facts of Life," from Dr. Sognnaes. You've heard the retiring Editor's "This Is Your Life" from Dr. Volker. You've heard the JOURNAL referred to as "a dead horse," "a live horse," and even as "a pig in a poke." Now let's begin to hear about it as a scholarly contribution to scientific literature! Moreover, if some of you don't think it a scholarly JOURNAL at present, let's try to make it one in the future.

Some of you here at the meeting have been asking whether there are going to be any changes made? Of course there are going to be changes made! One man never really *replaces* another—he merely takes up the good work and carries on—but never in exactly the same way.

What are some of these proposed changes?

1. The new Constitution states: "The Council shall appoint an Advisory Editorial Board of 10 or more members who shall act as consultants to the Editor." This has been done. The interim "Board of Editors" suggested over 50 members (most of whom are competent men in their respective fields) who were screened and reduced to 25 in number on the basis of academic disciplines and, secondarily, on the basis of geographic distribution (some from abroad have been included). It was gratifying that almost all of these men readily accepted when asked if they were available. Three, incidentally, gave an affirmative answer for a later year. The Publication Committee reviewed these names and added as well as subtracted a few. Yesterday, the Council appointed the men to the Advisory Editorial Board for 1 or 2 years. The regular term of service is 2 years, but to get underway an overlapping group is needed this first year. These 19 men will be listed in the April issue of the JOURNAL. The intention is to keep the membership of this group rather fluid; thus, each year some individuals will be reappointed, and some new men can be appointed to share the opportunity to review manuscripts and in other ways advise the Editor. Furthermore, I intend (like my predecessor) to send manuscripts to other referees—to any member of the Association or to nonmembers if a highly specialized topic needs reviewing. The *quality* of papers may be thus improved while the *quantity* may be decreased, perhaps proportionately.

2. The Publication Committee should study the incoming President's address of last evening and determine ways of quickly publishing short, research notes in the JOURNAL as suggested by Dr. Williams.

3. The Publication Committee should study the Charter presented by the St. Louis Section for its value in guidance of JOURNAL activities.

4. The Publication Committee should be thoroughly acquainted with all funds received for the JOURNAL on the one hand, and with the expenditures for JOURNAL publication on the other.

To encourage some immediate action on these proposals, I would like the Publication Committee to meet with the Council tomorrow, Sunday morning, at 10 in parlor E of this hotel to consider the several recommendations just listed.

Gentlemen (no ladies were present), this is your JOURNAL! It should continue to serve your needs in rapidly publishing meritorious investigative efforts in the broad stomatological field. I welcome your suggestions, all of which can help in formulating future editorial policy. But please be impressed with the fact that after all your advice

and opinions have been made available and have been considered by the Editorial Staff, I and the rest of the Publication Committee, of necessity, are the ones charged with making final decisions in the conduct of the JOURNAL. So be it, for now! I thank you.

FRANK J. ORLAND

H. MISCELLANEOUS

i. Statistics

Current membership . . . . 939 (774 members in North America; 165 members outside North America)

Attendance at 1958 meeting:	
Members attending	286
Newly elected members	19
Nonmembers	327
Guests	94
Total	726

ii. Necrology

Arthur Bulleid (England)	Samuel Charles Miller
Ernest A. Hooton	Thomas D. Speidel
Guy S. Millberry	L. R. Stowe

iii. Officers for 1958-59 year.

President	Ned B. Williams
President-elect	H. B. G. Robinson
Vice-president	Holmes T. Knighton
Honorary Vice-president	Edgar E. Coolidge
Secretary-Treasurer	Dan Y. Burrill
Asst. Secretary-Treasurer	Edward H. Hatton
Editor, JOURNAL OF DENTAL RESEARCH	Frank J. Orland
Publication Committee	John B. Macdonald
	David F. Mitchell
Councillors-at-large	Ralph C. Ireland
	Howard J. Merkeley

(Number of members voting—454)

IV. ABSTRACTS OF PAPERS "READ-BY-TITLE"*

T210. DEVELOPMENT OF TEETH IN SWISS ALBINO MICE.—*Dimitri Afonsky, Division of Dental Medicine, Colgate-Palmolive Company, in cooperation with Rutgers University, New Brunswick, N. J.* The development of molar teeth was studied in serial sections of heads obtained from 10 mice between birth and 17 days of age, and 53 gross specimens between birth and 35 days of age. The development of maxillary teeth precedes slightly that of the lower. The buds of the first molar (M1) and the second molar (M2) appear before birth, and that of the third molar (M3) between 5 and 7 days after birth. The beginning of apposition of dentin is observed: M1 2-3 days, M2 3-4 days, M3 9-11 days. Apposition of enamel begins: M1 3-4 days, M2 4-5, M3 10-12 days. The appositional growth of crown is completed: M1 7-9 days, M2 9-11, M3

*These papers are not presented in person although, where vacancies may occur in the program due to extenuating circumstances, one or more of these may be read by the principal author. These abstracts were listed by title in Section XX of Abstracts of Papers to be Presented at 36th General Meeting (*J.D.Res.* 37: 82, 1958) under the same numbers T210-T264, and in the Official Program of the 36th General Meeting under the numbers T213-T267. They are recorded in the complete "Index of Participants and Sequence Numbers for Abstracts" for the 36th General Meeting (*J.D.Res.* 37: 97, 1958) as well as in the index of "Read-by-Title" Abstracts appearing at the end of this section.

13-15 days. The formation of bifurcation begins: M1 9-12 days, M2 10-14, M3 14-18 days. The teeth first appear in the mouth: incisors 7-9 days, M1 12-15, M2 14-18, M3 2-24 days. All teeth are in occlusion by 27-30 days. The maxillary teeth are slightly larger than the lower, and measure: M1 1.75 by 1.0 mm., M2 1.25 by 1.0 mm., M3 0.5 by 0.5 mm. The distal slopes of the cusp tips of upper molars and the mesial slopes of the cusp tips of the lower molars are not covered by enamel.

T211. Caries Susceptibility in the Mouse.—*Dimitri Afonsky, Division of Dental Medicine, Colgate-Palmolive Company, in cooperation with Rutgers University, New Brunswick, N. J.* In our attempts to produce a caries-susceptible strain of Swiss albino mice, the animals used in our previous experiment (I.A.D.R. 35th General Meeting, 1957, Preprinted Abstracts, p. 67) were bred and 3 generations were obtained. Brothers were mated with sisters and the animals were kept on caries-producing diet, similar to that used before, and distilled water for periods from 167 to 180 days. In the first generation, 19.2 per cent of 26 males and 33.0 per cent of 21 females developed caries. Of the first and second molars, 5.5 per cent were involved. In the second generation, 50.8 per cent of 61 males and 59.3 per cent of 59 females developed caries. Of the first and second molars, 22.3 per cent were involved. In the third generation, 43.3 per cent of 53 males and 39.6 per cent of females developed caries. Of the first and second molars, 14 per cent were involved. The first lower molar was the most susceptible. The first upper and second lower were equally susceptible, and the second upper molar the least. No cervical, proximal, or smooth surface caries was observed. Caries frequently began in several sulci in one tooth.

T212. Effect of Desalivation in Reproduction.—*Dimitri Afonsky, Division of Dental Medicine, Colgate-Palmolive Company, in cooperation with Rutgers University, New Brunswick, N. J.* Two procedures were employed to study the effect of desalivation on reproduction in Swiss albino mice. Procedure I—mating of desalivated females with desalivated males of the same operated group. Procedure II—mating of completely desalivated females with normal males, and completely desalivated males with normal females. All animals were between 4 and 5 months old and 1 male was kept with 2 females for as long as 2 months. The operations were performed at weaning. The results of Procedure I were as follows: 10 out of 12 females in the control (non-operated) group conceived; in parotidectomized animals, 2 out of 6 females conceived; in females with submaxillary and sublingual glands removed, 1 out of 3; in completely desalivated animals (with three pairs of major salivary glands removed), 3 out of 13 conceived. The mean weight of animals in these groups was, respectively: females—31.6, 30.0, 17.6, and 23.6 Gm.; males—33.0, 33.6, 25.5, and 24.0 Gm. Procedure II gave the following results: out of 10 completely desalivated females mated with 5 normal males, 2 conceived; mating of 8 completely desalivated males with 16 normal females produced 2 litters, both by the same male. The weight of these animals was: desalivated males 33.0, females 27.5 Gm.; normal males 37.7, females 34.3 Gm.

T213. Citrate of Calcified Tissue.—*W. D. Armstrong and Leon Singer, University of Minnesota, Minneapolis.* The citrate uptake of powdered, dry, fat-free bovine cortical bone at pH 7.4 in 19 hour in vitro experiments has been determined. The effect of increasing the initial citrate concentrations of the bathing solutions from 0 to 30 mg. per cent results in an increase in citrate uptake by the bone. The calcium dissolved by the solutions at the end of the equilibrations remains constant despite variations in citrate content. Phosphate, which previously has been shown to be replaced on the

crystal lattice by citrate, increases in solution in a direct relation to the citrate uptake of the bone. The exchange of radiocitrate of the bone for that of the solution (labeled with C^{14} citrate) occurs at an initial rate of 3.3 per cent per minute. An increase in citrate:Ca ratio in the bone occurred with in vivo experiments with ethylene diamine extracted bone (anorganic bone-Losee) placed in the peritoneal cavity of rats for 11 days. This ratio approaches that for normal bone when cancellous bone is used. These results support the conclusion that the citrate of bones and teeth is derived from that of the body fluids.

T214. Fluoride Content of Sound and Carious Korean and Minnesota Teeth.—*Leon Singer and W. D. Armstrong, University of Minnesota, Minneapolis, and Myung Jim Park, Seoul National University, Seoul, Korea.* The determination of the fluoride content of the enamel and dentin of carious and noncarious individual teeth, obtained from patients over 30 years of age in Minnesota and Korea, is under way. This study was initiated because of the reputation, confirmed by our own observation of Seoul National University faculty members, that Koreans exhibit an unusually low dental caries attack rate. Preliminary results from the analysis of 8 noncarious and 17 carious teeth of Minnesota patients give an average per cent fluoride of 0.0089 and 0.0071, respectively. The enamel and dentin from 40 noncarious and 15 carious teeth from Korean patients have been separated and are now being analyzed. The enamel and dentin from 50 more noncarious and 20 more carious Minnesota teeth have been separated and purified. These specimens will be analyzed and the results reported.

T215. Addition of Bacteriostatic Agents to Acrylic Resins.—*Arthur N. Bahn, College of Medicine, Roger C. Michalsen, and Maury Massler, College of Dentistry, University of Illinois, Chicago.* Methyl methacrylate resins were found to be nonbacteriostatic once polymerization was complete when tested by the streak plate method, using *Streptococcus liquefaciens, Strep. viridans, Staphylococcus aureus, Staph. albus, Lactobacillus acidophilus, L. casei, Neisseria catarrhalis, Salmonella typhosa, Escherichia coli,* and *Candida albicans.* Addition of 10 per cent cadmium chloride, 10 per cent silver proteinate, or 10 per cent proflavine before polymerization yielded only slight bacteriostasis when standardized acrylic discs were tested by the agar pour plate method against a variety of microorganisms. Since these additives discolored the plastic, were potentially toxic at these concentrations, and were only slightly bacteriostatic, their use does not appear practical in dental resins. A mixture containing 3 per cent propylene glycol and 4 per cent substituted benzyl ammonium chlorides showed good residual bacteriostasis under the conditions of this experiment. The addition of 0.1 ml. of this mixture to 0.5 Gm. methyl methacrylate quick-setting polymer yielded plastic discs which exhibited a mean radius of the inhibition halo of 25.5 mm. when tested against *Staph. aureus.* After 10 days, the inhibition zone was 7.5 mm., after 20 days 4.5 mm., and on the thirtieth day, 2.5 mm. *Bacillus globigii* gave an initial inhibition zone of 35.5 mm., 15.0 mm. after 10 days, 8.5 mm. after 20, and 4.5 mm. after 30 days. This additive incorporated in acrylic also inhibited *Candida albicans,* as well as a mixture of salivary microorganisms. In vivo tests are now in progress.

T216. Effect of $CaHPO_4$ and NaF Dietary Supplements on Experimental Caries.—*Peter Barnard and Erling Johansen, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.* The purpose of this study was to determine the effect of 2 per cent $CaHPO_4$ and low concentrations of NaF

in the diet, separately or in combination, on the dental caries experience of 172 rats maintained on a highly cariogenic diet. Male and female rats, 25 days of age, were distributed among 6 groups. Each group (27-29 animals) received one of the following diets for a period of 85 days: Group I—diet 580 (66% sugar, 32% skimmed milk powder, 2% liver powder); Group II—580 + 2% $CaHPO_4$; Group III—580 + 10 ppm F^-; Group IV—580 + 2% $CaHPO_4$ + 10 ppm F^-; Group V—580 + 25 ppm F^-; Group VI—580 + 2% $CaHPO_4$ + 25 ppm F^-. Check analysis for the F^- content of each diet was made after mixing. The number of carious teeth, the number of carious lesions in smooth surfaces and fissures, and caries scores were determined. The mean number of carious lesions per rat were: Group I—51 ± 2.5, Group II—50 ± 3.7, Group III—20 ± 2.6, Group IV—27 ± 4.8, Group V—10 ± 1.3, and Group VI—13 ± 2.1. The addition of 10 ppm and 25 ppm F^- to either the 580 diet or 580 + 2% $CaHPO_4$ diet resulted in statistically significant reductions in dental caries. The addition of 25 ppm F^- was more effective than 10 ppm F^-. The 2% $CaHPO_4$ was ineffective in reducing dental caries and did not interfere with the protective action of low dietary concentrations of F^- in the highly cariogenic diet 580.

T217. Lymphoepithelial Lesions of Salivary Glands.—*Colonel Joseph L. Bernier, USA (DC), and Major Surindar N. Bhaskar, USA (DC), Armed Forces Institute of Pathology, Washington, D. C.* The lymphoepithelial lesions consist exclusively of epithelial and lymphoid elements. The multiplicity of names given to morphologically identical lesions of this group, and the application of same name to dissimilar lesions, illustrates the confusion in their classification and histogenesis. For example, the papillary cystadenoma lymphomatosum is known by about 20 other synonyms while the term adenolymphoma is used for both the Warthin's tumor and Mikulicz's disease. This investigation is based on histologic examination of serial sections and clinical analysis of 186 cases of lymphoepithelial lesions filed at the Armed Forces Institute of Pathology. It showed that (1) the parotid gland contains true lymph nodes within its substance; (2) both the parotid and cervical lymph nodes may contain glandular inclusions; (3) some lesions arise from the glandular inclusions within the lymph nodes. These are the papillary cystadenoma lymphomatosum, adenoma lymphomatosum, pleomorphic adenoma lymphomatosum, and solitary or multiple cysts; (4) lesions may arise which essentially represent hyperplasia of the intraparotid lymph nodes, but, in which, because of the local anatomic features, the parotid parenchyma is markedly involved. These lesions may be nodular or diffuse and are called the benign lymphoepithelial lesion of Godwin or sialolymphadenitis. In the past they have been called Mikulicz's disease. (5) The lymphoepithelial lesions as a group, contrary to the usual belief, are not confined to the parotid gland.

T218. Phosphatase Activity of Ligated Submaxillary Glands of the Rat.—*Theodore E. Bolden, Seton Hall College of Medicine and Dentistry, Jersey City, N. J., and J. P. Weinmann, University of Illinois, Chicago.* This investigation is based on biochemical assay of submaxillary glands of 31 male Sprague-Dawley rats, ranging in age from 125 to 150 days. The excretory duct of the right gland was ligated. Glands of untreated rats and the contralateral glands of animals with one ligated gland were used as controls. The animals were killed at 1, 3, 7, and 9 days postligation. Assays of alkaline and acid phosphatase activity were made on homogenates of frozen-dried glands. Alkaline and acid phosphatase activity varied within

a range of over 100 per cent between different untreated animals. The contralateral glands in the treated animals showed an even decline in alkaline phosphatase activity to approximately two thirds of the level preceding operation. The ligated glands showed a rapid progressive decline which reached its lowest point on the seventh day postligation. Activity at this time was about 10 per cent of the preoperative level. There was slight recovery on the ninth day. Acid phosphatase showed erratic changes with a rise on the first day in both patent and ligated glands followed by a slight progressive decline. Near normal values were recovered on the ninth day. When ligated glands were compared to contralateral patent ones, no demonstrable reduction in acid phosphatase activity was found.

T219. Incidence of Fractures of the Cementum in Human Dentitions.—*Theodore E. Bolden, Seton Hall College of Medicine and Dentistry, Jersey City, N. J., and Joseph P Weinmann, University of Illinois, Chicago.* This study is based upon the histologic examination of 71 human jaws obtained at autopsy from patients who ranged in age from 2 to 77 years. The jaws were fixed in formalin, decalcified in 5% HNO_3, divided into several blocks, embedded in celloidin, and cut serially. All sections were stained with hematoxylin and eosin. The 71 jaws contained 763 teeth (402 maxillary and 361 mandibular); 64 per cent of the jaws contained one or more teeth with one or more cementum fractures, 84 teeth (11 per cent) had cementum fractures. Sixty-two per cent of these were found in the apical third, 15 per cent in the middle third, and 23 per cent in the cervical third of the tooth. The fractures were almost evenly distributed between the mesial, distal, buccal, and lingual surfaces. The fragments of cementum varied widely in length. Some fragments were of cementum only, others were composed of cementum and dentin. Repair was indicated by the deposition of cellular cementum on the fragment of cementum and on the surface of the injured root.

T220. Effect of Flavored Beverages, Orange Juice, Sugar Solutions on Rat Caries.—*Michael Buonocore, Eastman Dental Dispensary and Erling Johansen, The University of Rochester, School of Medicine and Dentistry.* This study was undertaken to determine the caries-producing potentials of Coca-Cola, 7-Up, orange juice, and 10 per cent sugar solution when given as sole drinking fluids to albino rats placed on a nutritionally adequate, noncariogenic solid diet for 128 days. Weanling rats from several litters were distributed into 5 groups of 11 animals. Four groups received the beverages, and one group water plus the solid diet in which 10 per cent of the starch was replaced by sugar. Caries scores were obtained by a method similar to that used in hamster studies. The groups on the flavored beverages and sugar solution consumed approximately the same quantities of solid and liquid. The scores with standard deviations were as follows: 48.5 ± 12.9 for Coca-Cola, 39.6 ± 9.9 for sugar solution, and 16.0 ± 7.8 for 7-Up. The orange juice and solid sugar groups showed statistically significantly low caries scores of 0.0 and 2.2 ± 0.9, respectively. Sugar consumption for the solid sugar, orange juice, and flavored beverages, and sugar solution groups was in the ratio 1:2:4. Extensive erosion was present in the orange juice group, slight to moderate in the Coca-Cola and 7-Up groups, and none in the remaining groups. No consistent correlation was found between caries scores and pH or titratable acidity of the beverages. It is interesting that the 7-Up and orange juice, which produced low caries scores, also contain considerable amounts of citrate.

T221. The Comparative Efficacy of Gum, Toothpaste, and Mouthwash.—*Howard H. Chauncey, Tufts University School of Dental Medicine, Boston, Mass.*—Recent investigations concerned with the source of several of the

enzyme systems found in whole saliva have established that, for the most part, the enzymes found in whole saliva are derived from cellular debris and the oral flora. Studies concerning the cleansing ability of gum indicated that gum base and sweetened gum (sugar) base were capable of producing a 68 to 89 per cent reduction in hydrolytic enzyme titers of whole saliva. Although the latter compound was slightly more effective for the reduction of enzyme titers, there was no significant difference in the ability of the 2 compounds to reduce oral bacterial counts. The present investigation was undertaken to determine the comparative efficacy of gum, toothpaste, and mouthwash in the reduction of the hydrolytic enzyme levels of whole saliva. Sweetened (sugar) gum base was found to be less effective than mouthwash, but more effective than toothpaste. When the sweetened gum base (control) was compared to gum formulations containing both sugar and essential oils (flavoring agents), the latter were found to exert a possible bacteriostatic and/or anti-enzyme effect. Comparison of the flavored formulations with the toothpaste and mouthwash indicated that the presence of certain essential oils had an additive effect which increased the efficacy of the gum to a point where its over-all effect was superior to that of both the toothpaste and mouthwash.

T222. National Dental Evaluation of Children With Cerebral Palsy.—*M. Michael Cohen, Wm. A. Curby, and Manuel M. Album, Tufts University School of Medicine, and School of Dental Medicine, Boston.* In an investigation of 650 children from 9 states, the following data were compared: geographic location; state; which child of how many siblings; present age of parents (in 5 yearly groups); socioeconomic level; vitamin deficiency; malocclusion; vault; narrow arch; hypoplastic defect; gingivitis; bone loss; masticatory muscle tone; history of cerebral palsy in family; bruxism; parent-child relationship; mentality; additional congenital abnormalities; deglutitions; seizures and Dilantin therapy; attendance at school; mouth breathing; speech difficulties; drug therapy; type of cerebral palsy, spasticity, athetosis, rigidity, tremors, ataxia; prenatal disturbance; number of primary and permanent teeth present; decayed, missing, and filled teeth; orthodontic treatment; calculus; green stain; salivation; type of diet; blood Rh type; oral hygiene; patient cooperation; location and degree of gingival recession; location and degree of gingival enlargement; and type of x-rays available, none, intraoral, lateral jaw, lateral skull, other. The method employed in analysis of these children may be useful to national, state, and local programs in establishing dental clinics and private dental care for children with cerebral palsy. The extra dental evaluation may be helpful in clarifying etiologic factors associated with cerebral palsy.

T223. Acid Formation in Saliva and Plaques.—*H. Eggers-Lura, Laboratory of the County Hospital, Holbaek, Denmark.* Investigations into the content of lactic acid and pyruvic acid in saliva and plaque material have formerly been made with colorimetric methods which were rather inaccurate and unspecific. The new "enzymatic test method" (Boehringer Test Combinations, TC-B-C-G 15972-73-77) has been introduced for the first time in odontology and makes it possible to determine the content of lactate and pyruvate together with the lactic acid dehydrogenase activity, very exactly. It was found that the values given in previous investigations, using colorimetric methods, have been too high. By the enzymatic test method the author's previous results were confirmed, namely, that glycolysis and acid formation are more pronounced in the caries-resistant material that in the caries-active. Also, the dehydrogenase activity is as great in the caries-resistant as in the caries-active material and generally greater in the saliva than in the plaque.

T224. Problems of Experimental Polycythemia.—*Clark Eid and Robert J. Gorlin, School of Dentistry, University of Minnesota, Minneapolis.* Polycythemia, an increase in the number of circulating red blood cells, may be induced experimentally by several means: atmospheric anoxia, vasoconstrictors, pituitary erythropoietic factor, and soluble cobalt salts. The mechanism by which the cobaltous ion causes erythrocyte hyperplasia is not understood. A pilot study was carried out to determine the effect of polycythemia upon the structure of the head and neck and the dynamics of the pathologic process. Cobaltous nitrate (0.47%), at a level of 13.3 mg./Kg. daily, was administered intraperitoneally to 18 weanling white mice. Six controls were given a corresponding dose of sterile physiologic saline. At the end of 7 weeks, the experimental animals had an average of 14,420,000 red blood cells per cubic millimeter while the controls had but 9,700,000 red blood cells per cubic millimeter. The difference became less marked as the experiment continued. There was no difference noted in body size by 3 months, and no differences were noted in histopathologic section. A second pilot study employed 50 rats, equally divided according to sex. These were randomly distributed into groups of 13 experimental and 12 control animals. Red blood counts, hemoglobin values, white cell differential counts, and body weights were recorded weekly for 14 weeks and thereafter every 3 weeks. Cobaltous nitrate was similarly given at a level of 10 mg./Kg. This proved to be toxic for the rats, manifest by transient paralysis of the hind quarters; accordingly, the dose was reduced to 8 mg./Kg. By 6 weeks, polycythemia was evident, which reached its peak at 9 weeks. Hemoglobin reached its maximum at 7 weeks. Mean corpuscular hemoglobin was significantly less in the experimental group. Cobaltous nitrate retarded growth in the male experimental but not the female experimental animals.

T225. In Vitro Reactions of Epithelium to Growth Hormone Injected In Vivo.—*R. Gerstner, S. S. Stahl, O. Ioly, and E. O. Butcher, Murry and Leonie Guggenheim Foundation Institute for Dental Research, and New York University College of Dentistry, New York, N. Y.* Oral, vaginal, and anal mucosae of adult rats were injected subcutaneously with 0.5 mg. of growth hormone in 0.1 ml. distilled water 3 times weekly for 6 weeks. Controls were similarly injected with distilled water. The locally injected tissues were then removed, portions prepared for histologic study, and the balance transferred alive to culture flasks. The tissues were cultured for 14 days in Carrel flasks, in a coagulated medium of embryo extract, plasma, and physiologic saline. A fluid nutrient of dilute embryo extract and horse serum was changed at 2- to 3-day intervals. The growth hormone-injected oral mucosa showed the most extensive epithelial outgrowth. The cells were larger, more granular, and appeared earlier than epithelial cells of oral mucosa controls. Many had 2 nuclei which adhered to each other during migration. The cells also appeared earlier than connective tissue cells, reversing the growth sequence of these types in tissue cultures. Vaginal epithelium showed marked migratory activity, but individual cells did not differ greatly from controls, nor were binucleate cells observed. Anal tissues responded least, showing slightly more migratory activity than equivalent controls, and individual cell differences were not seen.

T226. The Willmar Story: A Pilot Study in Mass Free Oral Cancer Examination.—*Robert J. Gorlin and Lee Berglund, School of Dentistry, University of Minnesota, and Minnesota Division, American Cancer Society.* On Sept. 30, 1957, the Minnesota Division, American Cancer Society, performed a

pilot study in Willmar, Minnesota, a town of 7,000 people, to determine the effectiveness of mass free oral cancer examination in detecting oral and facial cancer. Other purposes of the pilot study were to determine the cost, required training time for the participating dentists, public response, professional (medical and dental) response, attitudes of various groups, etc. About 6 months elapsed between initial planning and actual program. Six hours of training by specialists in the field of oral cancer were given to the 15 participating dentists 2 weeks prior to the examination date. Numerous talks were given to lay groups and organizations by a field representative for the purpose of eliciting interest. Preregistration was attempted to determine the number of people who might be cooperative. Over 3,000 individuals signed up, registration being limited to those 21 years or over. A large auditorium was chosen and 15 partitioned booths constructed. The armamentarium was kept simple, consisting of mouth mirrors, tongue blades, finger cots, and pen flashlights. A few head lamps were employed by some men. All men brought a dental assistant to record findings on a prepared form. All information was returned to the patient's dentist and/or physician. Nothing was told the patient at the time of examination. Each examination, requiring about 3 minutes, consisted of visual examination of lips, tongue, buccal mucosa, oral floor, palate, oral pharynx, and face. The ostensible figures of pathologic findings were: Oral cancer—5, skin cancer—15, benign tumors—34. Follow-up is to be initiated on Dec. 1, 1957. The cost breakdown was $0.63 per person.

T227. The Trace Element Selenium and Its Influence on Dental Caries Susceptibility.—*D. M. Hadjimarkos, University of Oregon Dental School, Portland, Ore., and Carl W. Bonhorst, University of Portland, Portland, Ore.* A study on the selenium-dental caries relationship was conducted among children living in Jackson and Josephine counties, Oregon. A recent investigation revealed that children 14 to 16 years of age in these two counties showed a high prevalence of caries (Jackson county, 13.4 DMF teeth; Josephine county, 14.4 DMF teeth). Collection of 24-hour urine specimens was made from 79 white male high school children. Of these, 33 belonged to subjects from the high school of Medford, county seat of Jackson county. The remainder, 46, were collected from boys attending the high school of Cave Junction in Josephine county. Experimental work with laboratory animals has shown that a definite relationship exists between amount of selenium ingested and the excretion level in the urine. All of the urine specimens contained selenium. The mean urinary selenium concentration of children living in Jackson county was found to be 0.074 ppm and that of Josephine county 0.076 ppm. These values are regarded as being of considerable magnitude and show a definite selenium intake. The results of the present investigation, together with those obtained from our first study (*J. Pediat.* **40**: 451, 1952) among Oregon schoolchildren on the selenium-dental caries relationship, provide data which suggest the possibility that this trace element may be responsible for increasing caries susceptibility.

T228. Inhibition of Salivary Respiration and Fermentation by a Fluoridated Cationic Detergent.—*Ulrich Hermann and Hans R. Muhlemann, Dental Institute, University of Zurich, Switzerland.* Similar experimental caries reductions were found when stannous fluoride or diethanol-aminoethyl-N-ethanol-octadecylamine·dihydrofluoride ($\hat{=}$ E·2HF) was administered ad libitum in drinking water to Osborne-Mendel rats ($F^- = 0.1\%$). However, in the same experiment, the decrease in the rate of solution of intact

rat molar enamel in vivo was different in the 2 groups and more pronounced in the SnF_2-group. Attention, therefore, was drawn to an additional antibacterial cariostatic mechanism of the fluoridated cationic detergent (E.2HF). The oxygen uptake and anaerobic glycolysis of paraffin-stimulated human saliva to which E·2HF, E·2HCl, and NaF, respectively, were added at different F^- concentrations in aqueous solutions were determined by the Warburg method on 15 subjects. Inhibition of salivary respiration compared to controls was 88 per cent when using E·2HF at 10 ppm F^- and under otherwise identical conditions, 68 per cent when using NaF at 100 ppm F^-. The reduction of anaerobic glycolysis by E·2HF was 91 per cent at 3.2 ppm F^-, by NaF 65 per cent at 32 ppm F^-. Equimolar concentrations of E·2HF and E·2HCl showed a similar inhibitory effect on respiration and fermentation, indicating an antibacterial action due to the organic part of the molecule. Also, the onset of inhibition was acute with NaF and gradual with E·2HF or E·2HCl. Casein treated with 0.2 M E·2HF and thoroughly washed thereafter inhibited respiration and anaerobic glycolysis by 48 per cent and 65 per cent when added to the saliva-buffer-mixture. E·2HCl, in the casein adsorption test, was only active under anaerobic conditions (39 per cent inhibition).

T229. A Technic for the Filling of Root Canals in Hamster Molar Teeth.—*Robert B. Hoek, Emmett R. Costich, and James K. Avery, University of Michigan, Ann Arbor, Mich.* Previous studies in the replantation and transplantation of hamster molar teeth have indicated that necrosis of the pulp tissue may be a contributing factor in the failure of some of the transplanted and replanted teeth. In order to eliminate this problem, a technic has been devised for filling the root canals in hamster molar teeth via the apical end. The teeth are carefully removed, and a millimeter of the apical end resected with a fine diamond disk. The canals are probed via the apex with a fine smooth broach using a dissecting microscope to observe the area being treated. The canals are gently reamed with a No. 1 reamer and filed with a No. 1 file. Silver points are slightly modified by reducing the diameter of a No. 2 point on a cuttle-fish disk. The shape can be modified into an elliptical or a round one as the shape of the canal dictates. Canals are gently filled with Kerr sealer, and the points inserted with gentle pressure. Care must be taken during all operations in the root canal, for an excess of lateral or coronal pressure will result in a fracture of the root. The points are clipped off with scissors, gently burnished into place with a fine rotating stone, and a final polish of the apical end obtained with a very fine cuttle-fish disk. The teeth are then returned to their sockets, being careful not to depress them below their original level in the tooth socket.

T230. Effect of Vitamin E Deficiency on the Enamel Organ.—*J. T. Irving, University of the Witwatersrand, Johannesburg.* Most work in the past in this field has centered on the depigmentation of the rodent incisor in vitamin E deficiency, which is accompanied by a typical degeneration of the pigment containing ameloblasts, and it has seemed that the chief function of the vitamin was the protection of tooth color. More extended work shows that probably the entire enamel organ requires vitamin E for its maintenance. When animals are kept for longer periods on the diet previously employed by the present writer, the degeneration of the enamel organ may extend to the ameloblasts concerned with enamel calcification and, under these circumstances, the enamel matrix persists in an acid-insoluble form. The same results were obtained if the diet contained 2.5 per cent or 20 per cent cod liver

oil. The loss of tooth color is merely incidental to the degeneration of the enamel organ, the same being seen in vitamin A deficiency, and vitamin E plays no specific role in the metabolism of the pigment.

T231. Early Pulpal Calcifications of Permanent Teeth of Young Individuals.—*Verda Elizabeth James, University of Illinois, Chicago.* Histologic studies were made of 159 human permanent teeth extracted for orthodontic reasons from young individuals (11 to 25 years). Twenty-five of the teeth were unoperated and clinically intact prior to their extraction and 134 had cavity preparations and filling materials experimentally placed in noncarious areas for periods of time ranging from 15 minutes to 96 days. Early pulpal calcifications, described by Johnson and Bevelander (*J. D. Res.* 35: 714, 1956) as amorphous minute spheres or ellipsoids and called corpora amylacea by Hill (*J. A. D. A.* 21: 820, 1934), appeared in hematoxylin and eosin-stained sections as small, irregular basophilic bodies in the coronal pulp or upper root canal area of 90 (56%) of all of the teeth. Early calcifications were found in 13 (52%) of the 25 unoperated teeth and in 77 (57%) of the 134 operated specimens. These basophilic bodies were observed lying near and around blood vessels or entangled in nerve bundles. In 12 of the operated teeth, the calcifications were more localized near the experimental reaction. The incidence of these calcifications did not appear to be correlated with the different filling materials nor with the duration of the experimental procedure. Since the incidence of early calcifications in the unoperated teeth so closely approximated that in the operated teeth it would seem that these calcifications are of normal occurrence. The conditions under which these may be accentuated need further study.

T232. Histogenesis of the Keratinized Enamel Cuticle.—*Percy L. Johnson and G. Bevelander, Department of Histology and The Murry and Leonie Guggenheim Institute of Dental Research, College of Dentistry, New York University.* The utilization of subsequently decalcified celloidin blocks of tissue of the erupting pig's canine tooth, wherein tissue relationships are undisturbed, reveals the fact that Nasmyth's membrane is a product of the degenerating ameloblasts exclusively. There is no evidence for the existence of 2 cuticles as postulated by other investigators. The fact that the cuticle is of a keratogenous nature is confirmed by its affinity for Orange G of the Papanicolaou stain as well as the positive reaction to SH and SS groups recorded by the ferric-ferrocyanide test. The keratin nature of this structure is thereby confirmed and should no longer be referred to as a membrane in the strictest sense of the word. We suggest that the structure be referred to as the enamel cuticle or Nasmyth's cuticle.

T233. The Effect of Sodium Lauroyl Sarcosinate and Sodium Fluoride on Dental Caries in Rats.—*Paul H. Keyes and Carl L. White, National Institute of Dental Research, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Md.* One hundred and fifty newly weaned Sprague-Dawley rats were divided into 5 groups of 30 animals each and fed for 70 days the McClure-Folk diet 635 (*Proc. Soc. Exper. Biol. & Med.* 83: 21, 1956) consisting primarily of skim-milk powder (35%) and cornstarch (45%). Group I served as controls. Group II received 0.5 per cent SLS in drinking water; Group III, 50 ppm F^- as NaF in drinking water; Group IV, 0.5 per cent SLS in water during the last 35 days of the experimental period; Group V, 50 ppm F^- during the last 35 days. Stripped jaws were stained with $AgNO_3$ and hemisectioned to facilitate diagnosis of sulcal and proximal caries. The table below gives results.

COMPARISONS OF SIGNIFICANCE TESTS APPLIED TO PERCENTAGES OF MOLARS WITH CARIES ON VARIOUS SURFACES

	GROUPS	% OF TOTAL MOLARS CARIOUS	% OF MOLARS CARIOUS ON THE SPECIFIED SURFACE		
			BUCCAL	PROXIMAL	SULCAL
I vs.	II (SLS-70d)	N.S.*	Sig.	Sig.	N.S.
	III (NaF-70d)	Sig.	Sig.	Sig.	Sig.
	IV (SLS-35d)	N.S.	N.S.	N.S.	N.S.
	V (NaF-35d)	Sig.	Sig.	Sig.	Sig.

*N.S. = not significant; Sig. = significantly less.

Of special interest was the finding of a high incidence of sulcal and proximal caries after jaws were hemisectioned. Activity in sulci equaled or significantly exceeded that on smooth surfaces. These findings are at variance with those usually reported for these diets.

T234. DOUBLE MATING OF HAMSTERS TO ELUCIDATE GENETIC FACTORS INFLUENCING DENTAL CARIES SUSCEPTIBILITY.—*Paul H. Keyes and Johannes L. Scheltema, National Institute of Dental Research, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Md.* In our laboratory, phenotypically resistant and susceptible brown and albino hamsters are available. In susceptible animals, rampant caries, which usually involves 10 to 12 molar teeth, can be induced in albino animals in 35 days and in brown animals in 42 days or more. A susceptible albino female simultaneously mated to a brown and to a white male produces both brown pups and white pups. Thus, the sire is known from the color of the pups. All pups show susceptibility regardless of the caries history of the father. Resistant albino females simultaneously mated to brown and white males produce litters of variable susceptibility, apparently the result of uncontrolled transmissible factors. Back-crossing in either direction produces susceptible litters from susceptible mothers and variable litters from resistant mothers. These findings indicate the need for equalizing transmissible factors before genetic influences can be determined in hamsters.

T235. DIAGNOSTIC AND SCORING PROBLEMS RELATED TO EXPERIMENTAL RAT CARIES.—*Paul H. Keyes, National Institute of Dental Research, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Md.* New problems of diagnosis and scoring have resulted from the findings of dental caries on all surfaces of the rat molar dentition. This pattern of caries described by Stephan (*J. D. Res.* 30: 484, 1951) can be induced in almost all strains of rats raised at the N. I. H. by feeding high-carbohydrate low-fat diets. Jaws from animals fed the McClure-Folk diets 635 and 636 (*Proc. Soc. Exper. Biol. & Med.* 83: 21, 1956) and the highly cariogenic hamster ration (I.A.D.R. 35th General Meeting Preprinted Abstracts, p. 68) were examined to ascertain the pattern of caries induced and to provide material for developing a scoring system which would embrace all lesion types. After staining, all jaws were hemisectioned with a thin steel disc-saw. A simple linear scale has been applied to smooth surfaces and to theoretically flattened out sulcal areas. The extent of spreading of lesions is expressed in arbitrary units. Depth of penetration is recorded as: enamel only; slight, moderate, and severe dentinal. The pattern of caries and the incidence of lesion types varies considerably with different rat strains. Active carious lesions have been found on all molar surfaces. In jaws examined to date, more caries has generally been found in sulci than on smooth surfaces.

Thus, some sort of grinding or sectioning procedure is necessary for an examination of uniform susceptibility. The fact that several methods of scoring rat caries in current use here and abroad have somewhat similar features in common opens the possibility of developing an international method.

T236. The Influence of Altered Physical State of the Diet Upon Dental Caries.—*C. E. Klapper, and J. F. Volker, University of Alabama, School of Dentistry, Birmingham.* It has been observed that the cariogenic potentiality of a diet is definitely related to its physical nature. For example, desalivated hamsters fed only powdered whole milk will develop a moderate amount of dental caries. If this powder is reconstituted to fluid milk, practically no caries will result. The present investigation has been undertaken in an attempt to determine the factors responsible for this altered effect upon the etiology of dental caries. Similar groups of animals have been maintained on a cariogenic diet containing sucrose, whole wheat flour, powdered whole milk, salts, and alfalfa. The diet has been fed in the form of dry powder, paste, thick and thin slurry. Results indicate that the addition of only small amounts of water reduces greatly the cariogenic potentiality of this diet. Recently, a modified Hoppert-Webber-Canniff diet, containing 60 per cent sucrose, has been fed desalivated hamsters. Rampant caries develops in less than 60 days in most groups of animals, even those fed this diet in a thin slurry form. The study is continuing to collect data which will lead to an understanding of the factors responsible for the altered amount of caries which results when only the physical nature of the diet is changed.

T237. A Study of Ethylenediamine Treated Enamel.—*Donald E. Knapp, James K. Avery, Emmett R. Costich, School of Dentistry, University of Michigan, Ann Arbor, Mich.* Heads of young Rhesus monkeys were bisected sagitally and radiographed with a standard x-ray unit. The bicuspid teeth selected for this study ranged from those in which the enamel was first radiographically detectable to those just about to erupt. The teeth were removed and extracted with ethylenediamine. The samples were then immersed in water and microdissected with drawn glass probes. After extraction of the organic constituents from enamel with ethylenediamine, the enamel of the young developing teeth was so affected that the rods could be separated from one another in nearly their full length by careful microdissection. The enamel was found to be extremely brittle and great care was exercised in the separation of the rods. It would appear that the rods have calcified sufficiently to retain their morphology while the interprismatic substance is calcified to a lesser degree thus allowing separation of the rods. When isolated, the rods appear quite uniform in width and show clearly the periodic banding. The sides of the rods generally appeared smooth although some of the dissected rods displayed ragged edges. In those teeth where the enamel had attained its full thickness, attempts at microdissection were unsuccessful. Therefore, it would appear that the deposition of inorganic salts is relatively confined to the rod substance until approximately the full length of the rod is attained after which time the interprismatic substance also becomes sufficiently impregnated with inorganic salts so as to prevent microdissection of the rods. The mineralization of the interprismatic substance may be one of the stages in mineralization of enamel.

T238. Experimental Caries Inhibition and Simultaneous Enamel Solubility Reduction.—*Klaus G. Koenig and Hans R. Muhlemann, Dental Institute, University of Zurich, Switzerland.* The superiority of several organic fluorides over stannous fluoride in in vitro enamel protection (*Helv. Odont.*

Acta 1: 23, 1957) was not apparent in animal caries inhibition experiments, in which the inhibitors were administered in the drinking water. Saliva was assumed to have interfered, and the following investigations were performed: Four groups of weanling single-housed Osborne-Mendel rats received tap water and Stephan's cariogenic soft diet 580 ad libitum for 15 days. In each group, the following substances were applied topically to the molars, 10 seconds every day: (1) stannous fluoride, (2) cetylamine-hydrofluoride, (3) diethanol-aminoethyl-N-ethanol-octadecylamine·dihydrofluoride (E·2HF), and (4) H_2O (control), the F^- concentration being 0.1 per cent, the pH 3.1 ± 0.1. At the end of the experiment, the crowns of the first and second molars were exposed to a pH 4 phthalate buffer for 1 hour. The removal of phosphorus was determined. Afterward, the same jaws were sectioned serially for microscopic caries scoring. Initial enamel fissure lesions (score A) were distinguished from lesions involving the dentinoenamel junction (score T) and from definite dentin lesions (score B). The results are assembled in the table below. Caries inhibition was similar for SnF_2 and E·2HF; however, the reduction in solution rate of the intact molar enamel in vivo was most pronounced in the SnF_2-group. The experimental arrangement presented may be used in order to better understand the mechanism of caries inhibitors.

INHIBITOR	NO. RATS	AVG. REMOVAL OF PHOSPHORUS ± S.E. MG. PER 100 ML.	PERCENTAGE SOLUTION RATE REDUCTION	AVG. NUMBER OF LESIONS		
				A	T	B
SnF_2	10	0.73 ± .05	43	8.0	2.1	0.8
Cetylamine · HF	10	0.94 ± .05	26	9.1	3.2	1.4
E · 2HF	12	1.05 ± .06	17	7.1	3.0	0.08
H_2O	11	1.26 ± .06	–	11.2	8.8	4.5

T239 In Vitro Electrical Resistance Measurements of Teeth With and Without Silicone Film. *M. Krakowski, B. S. Savara, M. M. Phatak, University of Oregon Dental School, and J. Dement, Dement Research Laboratories, Portland, Ore.* Application of silicones to enamel surface may prevent cariogenic activity. Electrical resistance measurements were made to detect the presence of silicone film on teeth, specific resistivity of the silicones being about 10^{14} ohm-cm., and that of teeth about 10^6 ohm-cm. Liquid contact in place of solid metal electrodes gave consistent readings, whereas metal electrodes tended to push away the surface silicone film. The crown of the tooth was immersed in saline solution which served as one electrode, and a copper wire inserted through the root canal into the pulp chamber served as the other electrode. Circuit was completed by a commercial micro-ammeter and a 1.5 volt dry cell. Amperage was read directly and resistance calculated. Five control and five experimental readings, after the application of silicone film, were made on each of thirteen teeth. The mean control resistance was 0.64×10^5 ohms. The mean experimental resistance of the teeth was 0.71×10^5 ohms. The mean of the difference between experimental and control readings was 0.057×10^5 ohms with a range of -0.13 to 0.17×10^5 ohms. For 95 per cent confidence limits, the true mean difference lies between 600 and 10,800 ohms and is statistically significant. The small difference in resistance is probably due to the microthinness of the silicone film.

T240. Effect of Ultrasonic Cavity Preparation on the Periodontium on Dogs.—*Harry Lyons, John J. Salley, and George W. Burke, Jr., Medical College of Virginia, Richmond, Va.* A preliminary investigation was carried out to ascertain the response of the periodontal tissues to cutting cavity

preparations with an ultrasonic device. Four adult dogs were anesthetized and Class V labial and buccal cavity preparations were made in all teeth of the left quadrants of the maxilla and mandible except the last molars where Class I occlusal preparations were cut. As a control measure, the upper and lower right quadrants of each animal were subjected to an identical procedure but with an inverted cone carbide bur in a conventional rotary handpiece as the cutting instrument. Class V cavity preparations were used in the majority of teeth in order to evaluate the effect of lateral stress on the periodontium. All cavities were filled with zinc oxide and eugenol. Dogs were sacrificed at 3 hours, 1 day, 2 days, and 4 days, respectively, and block sections of each tooth were prepared by the celloidin-paraffin technic. Every tenth section was mounted for study. Histologic examination has failed to reveal any discernible differences between the cementum, periodontal membrane proper and alveolar bone on sides of the jaws prepared with the ultrasonic device and those of the opposite side prepared by the conventional technic.

T241. Endamoeba Gingivalis and Its Relation to Gingival Pathology.—*Maurice A. Mazzarella and Noshi Monsour, Naval Medical Research Unit No. 3, Cairo, Egypt.* The "MIF" preservative stain for intestinal amoebae developed at No. 3 by Sapero, Lawless, and Stome was used to study *Endamoeba gingivalis* in an attempt to relate oral conditions to infestation with the parasite. Complete oral examination was made of 50 patients, both American and Egyptian, who presented themselves for treatment. Every clinically discernible pathologic condition of the teeth and gingival tissues was recorded. Patients were of both sexes and all ages. In order to obtain data on mode of transmission, they were from the same family whenever possible. Samples were taken of both saliva and gingival debris. No relation could be seen to supra- or subgingival calculus, materia alba, or decay incidence. Amoebae were present in all cases of periodontoclasia that showed purulence. This agrees with the accepted theory that *E. gingivalis* plays the role of a seavenger of dead bacteria and dead leukocytes. However, the parasite was found in most patients who had any generalized pathologic condition of the gingivae such as hemorrhagic inflammation, gingivitis, and generalized periodontoclasia. Of all the patients studied, 36 per cent showed generalized gingival pathology with the presence of *Endamoeba gingivalis*, while 8 per cent showed amoebae without generalized gingival pathology. This difference was significant at the 1 per cent level of confidence.

T242. The Local Effect of Insulin on Wound Healing in Rats.—*Govind B. Shankwalker and David F. Mitchell, Indiana University School of Dentistry, Indianapolis.* Previous evidence shows that the topical application of insulin on the gingiva produces increased keratinization of the epithelium, increased fibrosis, and an increase in amount of gingival glycogen. As these findings were suggestive of beneficial effects on the healing of soft tissue wounds, an experiment was conducted to study the local effect of zinc insulin on the healing of soft tissue wounds in rats. A total of 20 male albino rats were divided into 4 groups. Four horizontal incisions, approximately 1 inch in length and 1 inch apart, were made on the back of each rat. In 2 wounds, 10 to 15 mg. of the zinc insulin powder (Lilly) was gently inserted. In the other 2, an equivalent amount of sodium chloride was inserted to serve as a control. These groups were sacrificed at periods of 2, 5, 7, and 10 days. In the first 3 groups, one specimen from each of the rats was utilized for measuring the tensile strength of the wound. Histologically, the experimental group showed greatly accelerated wound healing over the controls. This

was seen more markedly after the 5-day healing period. Accelerated epithelization was evidenced by an increase in thickness, length, and cellular differentiation. The connective tissue showed greatly advanced healing as observed by increased numbers of fibroblasts, collagen fibrils, blood vessels, and a reduced amount of inflammatory reaction. Measurement of the tensile strength of the healed incisions also showed similar differences. This was observed more particularly in specimens of the 5- and 7-day periods. It appears from this experiment that insulin exerts a beneficial local effect on the healing of surgical incisions in rats, as compared to locally applied sodium chloride.

T243. Fluoride Tolerance: Action of Fluoride on Weight, Calcium Metabolism, and Calcification of the Bones and Teeth.—*J. C. Muracciole, Hospital Nacional de Odontologia, Buenos Aires, Argentina.* Increasing doses of fluoride, as NaF, were administered intraperitoneally to 105 rats divided into 21 groups. The doses ranged from 0.036 to 2.26 mg. F^- per day. The peritoneal route was chosen because it ensures administration of the entire dose. The amounts used were within the limits of tolerance by the rat but are useful in prevention of dental caries. Administration of fluoride for 90 days did not lead to any changes in weight, development, teeth, or calcium metabolism. The long bones showed greater calcium fixation than in animals not receiving fluoride. The latter studies were done with Ca_{45}. These results indicate that the doses employed here can be used for prophylaxis of dental caries without affecting physiologic mechanisms.

T244. Changes of Keratins Under Load.—*H. H. Neumann, Columbia University, New York, N. Y.* The organic matrix of the enamel contains a spongelike network of colloids into which the crystallites are laid down. The enamel is developmentally related to the epidermis and, since posteruptive changes in the enamel keratins are difficult to explore, one may observe the behavior of the related keratins in the epidermis and apply the findings with caution to the enamel. Pressure is the most powerful agent influencing the chemicophysical characteristics, and the state of hydration of the keratins in the skin, as witnessed by the adaptation in the cornification of the epidermis, under loads. Histologically the changes in keratins, depending on whether they have been subjected to pressure, may be demonstrated. Hooves of animals are softened by disuse, that is, lack of compression, and the keratinization of the soles of the feet of natives walking barefoot on rugged terrain, is rapidly modified by wearing shoes. The process is reversible. The degree of keratinization in the skin is responsible for its resistance to harmful chemical and physical agents. Furthermore, keratin, being an amphoteric protein, has the ability to neutralize acids and alkalis. It controls ionic diffusion and the penetrability of the skin depends on the state of the keratins. If the keratins in the enamel behave similarly, the protection which they afford to the imbedded crystallites would be explained. Similarly, its dependence on the occasional transmission of higher loads—as in native diets—to enhance its protective properties by reducing the hydration of these enamel colloids would be explained.

T245. A Quantitative Method for Screening Nutritional Symbionts.—*Thomas A. Nevin, National Institute of Dental Research, T. Shiota and Edward G. Hampp, American Dental Association, National Institute of Dental Research, National Institutes of Health, U. S. Department of Health, Education and Welfare, Bethesda, Md.* An auxanographic assay method has been adapted for both qualitative and quantitative measurements of the ability of any given microorganism to supply required nutrilites to an indicator organism. The

assay medium, deficient in any one of several growth factors, is seeded with the indicator organism and poured into sterile Pyrex glass dishes. Standard reference solutions are pipetted to 1.5 cm. filter paper pads. Standardized suspensions of washed reactant organisms are similarly dispensed. After drying in a desiccator, the pads are placed on the surface of the seeded medium. The reactant organisms are tested for ability to grow and the production of the limiting factor as a function of growth, by placing a small number of cells directly on the surface of the seeded medium with a bacteriologic needle. Quantitative measurement is achieved by measuring and comparing the zones of growth which develop around the pads after adequate incubation. For example, *Streptococcus salivarius, Streptococcus fecalis R* and *Pediococcus citrovorum* have been used as indicator organisms to show that *Candida albicans* has activity equivalent to 0.2 μg pantothenate and 0.1 μg riboflavin. A type strain of *Escherichia coli* has activity as 0.4 mμg citrovorum factor, 4 mμg folic acid, 0.2 μg pantothenate, and 0.1 μg riboflavin.

T246. Hamster Gingival Plaque and Dodecyl Benzene Sulfonate.—*Paul B. Risk and David F. Mitchell, Indiana University School of Dentistry, Indianapolis.* In a previous study it was concluded that the gingival plaque in the hamster (*J. D. Res.* 35: 651, 1956) consists in large part of aciduric microorganisms, and it was shown that the plaque could be eradicated by feeding the animals penicillin for 2 weeks. This test for a possible caries inhibitory drug was applied to dodecyl benzene sulfonate. Fifty, old, plaque-positive Syrian hamsters which had been maintained on a high carbohydrate diet for many months were selected and divided into a control and experimental group. Each animal had a caries score on gross observation in vivo of 4 or more palatal gingival surfaces involved (6 possible, one opposite each upper molar). Both groups were fed a high carbohydrate diet (CPDS), food and water ad libitum for 2 weeks. The experimental group received, in addition, 1 per cent by weight of dodecyl benzene sulfonate (recommended by R. S. Manly on the basis of in vitro salivary sediment studies) in the dry diet. The animals were examined at 2 weeks and showed the following differences:

	Control	*Experimental*
Average weight change	0.1 Gm.	–6.5 Gm.
Average plaque score	4.5	5.5
Average diet eaten	5.7 Gm./day	5.8 Gm./day

This, we believe, is a measure of the lack of influence of dodecyl benzene sulfonate on the bacterial population of the gingival plaque of the hamster maintained on a high carbohydrate diet.

T247. Further Studies of the Effect of Tobacco on Oral Tissues.—*John J. Salley, Medical College of Virginia, Richmond, Va., and Seymour J. Kreshover, National Institute of Dental Research, Bethesda, Md.* Previous investigations relating to the prolonged application of tobacco in the form of whole cigarette smoke to the lips of normal and gonadectomized mice and mice on vitamin B-complex deficient and adequate diets have failed to produce any significant changes. Desalivated and nondesalivated, normal and gonadectomized and vitamin B-complex deficient hamsters have likewise exhibited no demonstrable alterations in the palatal mucosae after extended periods of smoke application. On the other hand, the ears of mice and, to a lesser extent, hamsters have presented findings indicative of hyperplasia, ulceration, and dyskeratosis subsequent to prolonged smoke application but only when the animal was concurrently suffering from vitamin B-complex

deficiency. On the basis of observations on the ears it was felt that further study of the effect of tobacco on an oral epithelium which is somewhat removed from the intraoral environment was indicated. The hamster pouch which has proved to be susceptible to action of known carcinogens was chosen for this investigation. The pouches of 40 albino hamsters were subjected to daily application of a controlled volume of cigarette smoke for a period ranging up to 22 months. Histologic examination of these tissues has exhibited no significant changes other than slight epithelial hyperplasia in 3 animals. The results of this and previous studies would point to the conclusion that whole tobacco smoke per se seems to have no adverse effect on the oral mucosa of the experimental animal.

T248. Effect of Thyroxine and Triiodothyroacetic Acid on Incisor Eruption Rate.—*Sheldon Schumer and Herbert Wells, Harvard School of Dental Medicine, Harvard University, Boston.* The effects of the thyroid hormone, L-thyroxine (T_4), and one of the newer active synthetic analogues, L-tri-iodothyroacetic acid (TA_3), on the opposed and unopposed eruption rates of the rat incisor were investigated by the method of Bryer. This method involves a surgical shortening of the left mandibular incisor to remove it from functional occlusion. The unopposed eruption rate was measured at intervals of approximately 48 hours. Five control measurements were obtained for each rat before beginning treatment. The 2 substances were administered daily by the intraperitoneal route in doses estimated to be equipotent by other criteria, T_4 at 50 μg and TA_3 at 250 μg/100 Gm. After 14 days, the mean unopposed eruption rates were as follows: control, 0.815 mm./24 hr.; T_4, 0.885 mm.; TA_3, 0.900 mm. This demonstrates a highly significant effect ($P < .01$) of both substances over the controls. However, the mean opposed eruption rates measured on the right mandibular incisor were not significantly increased under these conditions. These results show that the synthetic thyroid hormone (TA_3) as well as the natural hormone (T_4) can stimulate the unopposed eruption rate and support Bryer's contention that measurement of the unopposed eruption rate provides a more sensitive indicator of changes in eruptive activity than measurements with the teeth in occlusion.

T249. Effects of Burs, Airbrasive and Ultrasonic Devices on the Pulp of Rat Incisors.—*Michel Serfaty, Maury Massler, and Verda E. James, University of Illinois, Chicago.* Cavities were prepared in the upper right incisors of 69 albino rats using burs rotated at 6,000 r.p.m., airbrasive or ultrasonic devices. The cavities were filled with Aquadont, or zinc oxide and eugenol. The animals were sacrificed 7 days later, and routine histologic preparations were made of the experimental teeth. Under shallow cavities prepared by burs, the pulp showed no significant damage. Under medium and deep cavities, damage to the odontoblasts and inflammatory changes in the pulp increased in severity approximately in proportion to the depth of the cavity. Under cavities prepared by the airbrasive and ultrasonic devices, damage to the pulp was not so clearly related to cavity depth. Severe damage was noted under 30 to 50 per cent of the shallow cavities while little or no damage was observed under 20 per cent of the very deep cavities. When damage to the pulp did occur under deep cavities prepared by the airbrasive or ultrasonic device it was characterized by the extensive formation of osteodentin within the pulp. The reason for this is not clear. It was concluded that while the airbrasive and ultrasonic devices did not necessarily produce more damage to the pulp than did the bur, they also did not produce less damage, under either shallow or deep cavities, in rat incisors.

T250. Response to Despeciated Calf Bone and Calcium Hydroxide Implants.—*Govind B. Shankwalker and David F. Mitchell, Indiana University School of Dentistry, Indianapolis.* A study of the influence of despeciated calf bone (Osteogen, Squibb) and calcium hydroxide on the regeneration of surgically resected alveolar bone in adult dogs was carried out. Forty-eight defects in 16 quadrants of the 4 dogs were created by raising a gingival flap and resecting a block of bone approximately 6 by 5 mm. Sixteen of the defects were filled with calf bone, 16 with calcium hydroxide, and the remaining 16 were left unfilled to serve as controls. Specimens for histologic study were obtained at the experimental periods of 18, 30, 43, 65, 98, 125, 145, and 158 days. Grossly, there was no visible bone regeneration in any case. Histologically, both the implants were tolerated by the host tissue without any foreign body reaction. Slight osteogenic influence was observed in the area of both the implants, however, slightly better results were obtained with calf bone than with calcium hydroxide. Local inflammatory reaction emanating from the gingival sulcus seemed to have influenced the results greatly. Surgical bur holes in the molar regions which were allowed to heal without any communication with the oral cavity showed a better response, as evidenced by greater osteogenic activity. More favorable influence on the healing process of the wound area was observed in cases of calf bone implants. This was evidenced by less inflammatory reaction and better organizing influence on the other detached supporting tissues. Calcium hydroxide, however, seemed to show better influence on the deposition of new cementum in the detached area.

T251. Sodium and Potassium Levels of Saliva and Salivary Glands Following Prolonged Stimulation.—*Ira L. Shannon, School of Aviation Medicine, USAF, Randolph Air Force Base, Texas.* Thirteen male subjects chewed 5 large (size 32) rubber bands for a 3-hour period, thus providing 36 5-minute mixed saliva samples. Mean volume for all subjects ranged from 7.1 to 8.7 ml., there being no systematic increase or decrease in rate of flow over the 3 hours. Mean sodium values (range 10.2-18.3 mEq./L.) tended to decrease with time of stimulation, while mean potassium values (range 20.5-24.0 mEq./L.) tended to increase as the stimulation continued. Homogenate assays were carried out on 198 submaxillary glands from Cotton rats, Wistar rats, chinchillas, and rabbits. Varying doses of pilocarpine were administered over periods of time up to 2 hours. Alterations in mean values for all treated animals when compared to controls were as follows:

	Sodium	*Potassium*
Cotton rats	+36.6%	–42.5%
Wistar rats	+60.4%	–47.9%
Chinchillas	+49.3%	–20.6%
Rabbits	+46.6%	–33.8%

These findings suggest that there is an abundant, readily available source of sodium for the working gland and that such is not true to the same degree for the potassium ion. Histologic studies are being carried out.

T252. Salivary Sodium, Potassium and Chloride Levels in Subjects Classified as to Caries Experience.—*Ira L. Shannon, Research Dentistry Division, School of Aviation Medicine, USAF, Randolph Air Force Base, Texas.* Flame photometric and titrimetric analyses were performed on saliva samples collected from 537 selected male subjects. "Carious" subjects presented at least 20 carious surfaces ($\bar{x} = 48.8$) and no restorations of any type; "resistant" participants presented no carious lesions of any type; "resistant" participants

presented no carious lesions and no restorations; "restored" cases had at least 20 ($\bar{x}$ = 40.6) surfaces restored with silver amalgam and had no evidence of dental caries. To qualify, the subjects were required to be free of periodontal involvement and to have at least 20 ($\bar{x}$ = 28.2) permanent teeth present. The data were analyzed to determine if significant differences existed in the levels of volume, sodium, potassium or chloride for the 3 subject groupings. No significant differences were found in volume or potassium levels. Mean sodium concentration was significantly lower ($P < .05$) for the "resistant" group than for the other 2 categories. The mean chloride value for the "resistant" group was significantly lower ($P < .05$) than the mean level for the "carious" subjects. A similar but even more significant ($P < .01$) difference was found between the chloride means of the "resistant" and "restored" subjects. No significant differences were noted between the "carious" and "restored" groups. There was an extensive overlap in values for individuals throughout the 3 groups. Predictability of an individual's dental status based upon levels of these variables would be, at best, extremely low.

T253. The Temperature of Teeth and Gingiva.—*Max Shapiro, California Institute of Dental Research and Cedars of Lebanon Hospital, and Ben H. Ershoff, Western Biological Laboratories, Culver City, Calif., and Southern California School of Medicine.* The object of the study was to determine the temperatures of normal teeth, and the temperatures of gingiva when normal and inflamed. A Thermistor was designed with minimum mass and so that it would be temperature sensitive at one point only. It is accurate to 0.01° C. Human beings were selected and measurements taken from the surface of 9 areas of each tooth, and 3 areas in the attached gingiva, all on the labial and buccal surfaces. Teeth were found to be unequal in surface temperature, with the warmest part of the tooth being the upper middle ninth. Different teeth had differences in temperature, varying with their position in the arch. The temperature was higher when inflammation was present in the gingiva, and lower when inflammation subsided, in the same patients.

T254. The Relationship of Nonaxial Forces in Deglutition to Periodontal Disease.—*Irving M. Sheppard, Dental Department, Montefiore Hospital, New York, N. Y.* The frequency of occlusal contact in deglutition led to our investigation of the lateral forces exerted during this procedure. Twenty patients between the ages of 40 to 75 were studied. Ten showed evidence of severe periodontal disease as denoted by increased gingival crevice depth and a loss of alveolar crest bone. Ten patients presented virtually no such evidence. Duplicate study models were made in each instance and occluded in centric position. One set of models was then ground in a vertical plane, simultaneously through both right and left molars, forward to the cuspid region. Photographs were taken at 2 areas of each molar relationship, and one with each of the bicuspids. The duplicate models were ground in a somewhat sagittal direction from molars to cuspids, through the buccal cusps. The presence of nonaxial forces exerted in a buccal or lingual direction, as evidenced by cross-sectioned models in centric position, appeared associated with periodontal disease. Nonaxial forces in a mediodistal direction were also found associated with such pathology. Sectioned models of patients without periodontal disease showed essentially axial forces in both buccolingual and mesiodistal sections.

T255. Reaction of Orange-G With Bone and Dentin Collagen.—*C. C. Solomons, Joint Dental Research Unit of the Council for Scientific and Industrial Research and the University of the Witwatersrand, Johannesburg,*

South Africa. Combination of dye with the proteins of ox bone and human dentin and demineralization of these tissues were carried out simultaneously. Known weights of tissue containing 5 mg. of protein were placed in 2 ml. of 0.1 per cent Orange-G aq. in 50 ml. conical flasks containing 10 ml. of 20 per cent citric acid. The flasks were shaken for 8 hours at room temperature and allowed to stand. At intervals of 24 hours, the supernatant solutions were transferred to 25 ml. standard flasks and made up to volume with distilled water. The amount of dye combined with the basic protein groups was measured by recording the optical density of the solution at 480 mμ on the Beckman spectrophotometer and comparing this value with that obtained from the same dilution of the dye which had not been placed in contact with protein. The amount of dye bound to protein was expressed in microequivalents per gram of protein and plotted against the percentage dissolution. In bone and dentin it was found that as demineralization in citric acid progressed, the number of microequivalents of Orange-G bound per gram of protein increased in a linear manner from 20 in the fully calcified tissues, to a maximum of 336 at complete demineralization, when plotted against the percentage dissolution of mineral materials. By treating these tissues with dinitrofluorobenzene during demineralization similar values for the number of reactive ε-amino groups have previously been obtained. Thus, it is thought that basic groups of bone and dentin collagen may play a part in the combination of mineral salts to the organic matrix.

T256. Organic Content of Enamel From Greek and British Teeth.—*M. V. Stack and J. Halazonetis, Dental School, University of Britsol, England.* Marked Differences in enamel organic content are indicated by analytical data reported on mineral components of Greek and American teeth (Asgar, *J. D. Res.* 35: 742, 1956). Enamel samples from pumice-cleaned caries-free Greek and British premolars have been purified by the same method. Organic contents showed the same mean values (about 0.6 ± 0.1 per cent) as have been noted for large groups of enamel samples from American and British teeth.

T257. The Effects of Somatotropic Hormone on the Periodontal Structures of Normal Adult Male Rats.—*S. S. Stahl, The Murry and Leonie Guggenheim Foundation, Institute for Dental Research, and the Department of Periodontia and Oral Medicine, New York University College of Dentistry, New York.* Twenty-nine Long-Evans strain male rats, weighing an average of 225 grams at the beginning of the experiment, were divided into 3 groups. Fifteen animals received 0.5 mg. of STH in 0.1 ml. of distilled water, buffered to a pH of about 10, injected into the mucobuccal fold 3 times weekly for a total of 6 weeks. Nine control animals received buffered distilled water injections in the same manner as outlined for the hormone-treated group. Five animals were kept as untreated controls. At the end of the experimental period, the maxillae were dissected out, decalcified, and prepared for histologic study using hematoxylin-eosin stain and silver impregnation. Hormone-treated animals showed an increase in the cellularity of the periodontal membrane, increased cementum formation, and a marked increase in endosteal alveolar bone formation. The newly deposited bone contained fewer osteocytes and did not show lamellar organization. In many areas, the newly formed bone encroached upon the marrow spaces reducing them markedly. Osteogenic activity could also be observed at the periodontal surface of the alveolar bone. However, this was not as prominent as the endosteal bone formation. The newly formed bone stained lighter with silver impregnation and the fibers appeared to be less organized than those seen in the alveolar bone of the control animals.

T258. Effects of Protein Deprivation on the Periodontium of Young Adult Male Hamsters.—*S. S. Stahl, S. C. Miller, and E. D. Goldsmith, The Murry and Leonie Guggenheim Foundation, Institute for Dental Research, and the Departments of Periodontia and Oral Medicine and Histology, New York University College of Dentistry, New York.* Seventy-four young adult male hamsters, weighing an average of 106 Gm. at the beginning of the experiment, were divided into 3 groups. Animals in Group I were placed on a normal Purina Chow Checker diet. Animals in Group II were placed on a normal granular Purina Chow Meal diet. Animals in Group III were placed on a powdery protein-free diet. A number of animals in each group were sacrificed after 60 days, the remainder after 120 days. Some animals in Group III received a normal diet for 60 days following 60 days of protein deprivation. Histologic observation of the periodontal tissues showed that protein deprivation caused endosteal osteoporosis with a reduction in cellularity of the periodontal membrane. The epithelial attachment level did not appear to be affected. The intake of a normal granular diet caused marked interproximal food impactions which were associated with downgrowth of the epithelial attachment, resorption of the interproximal alveolar crest, and some evidence of inflammatory infiltrate in the area. The periodontal structure of the maxillary molars showed more severe periodontal lesions than did the periodontium of the mandibular molars. Restitution of a normal diet following protein deprivation reduced the endosteal osteoporosis and increased the cellularity of the periodontal membrane.

T259. Pulpal Damage Under Extracoronal and Intracoronal Preparations.—*Stanley D. Tylman, John M. Spence, Marvin B. Weiss, and Maury Massler, University of Illinois, Chicago.* Jacket crown preparations were made on 7 anterior teeth using diamond discs and diamond stones revolving at 6,000 r.p.m. and 20,000 r.p.m., with and without a water-spray coolant. One side of each tooth was cut at lower speed, and the other at the higher speed. Additionally, shallow, medium, and deep inlay preparations were made in 72 teeth using diamond stones and burs at speeds ranging from 3,000 to 70,000 r.p.m., with and without a water spray. All preparations were covered with a zinc oxide–eugenol paste. The teeth were extracted 48 hours or 7 days later and subjected to routine histologic preparation and analysis for pulpal changes. Shallow but extensive jacket-type preparations resulted in little or no pulpal damage. The changes were not significantly different from those observed under much less extensive shallow inlay preparations. Pulpal damage was progressively more severe as the inlay preparations became deeper. Absence of coolant had no significant effect under the jacket preparation or shallow cavities but resulted in more severe pulpal damage under deep cavities. Pulpal damage was more severe under cavities prepared above 16,000 r. p. m. than those prepared under 6,000 r. p. m. It was concluded that pulpal damage in human teeth was more directly correlated with cavity depth than with extent or width of the preparation.

T260. Incidence of Parasitic Protozoa in the Human Mouth.—Wayne *W. Wantland, John W. Remo, Illinois Wesleyan University, and D. L. Winquist, Dental Clinic, Bloomington, Ill.* Wet smears prepared from samples of material obtained by scraping the base of the teeth and gingivae of 100 patients, 13 to 70 years of age, revealed the following protozoan parasites: *Endamoeba gingivalis,* 50 per cent; *Trichomonas tenax,* 34 per cent. Eighteen patients carried both parasites. *Leishmania braziliensis* (leptomonad forms) were observed in a sample taken from the mouth of a woman 48 years of age

who gave a history of recent travel in Mexico and the Panama area. Cultures in NNN media yielded various phases of longitudinal division of these flagellates and varying shapes with typical leptomonads most numerous.

T261. Color Reaction Between Auric Chloride and Diiodotyrosine.—*Joel Wisotzky and Joan D. Bell, Division of Dental Medicine, Colgate-Palmolive Company, in cooperation with Rutgers University, New Brunswick, N. J.* It was found by oxygen uptake studies, using the Warburg manometric technics, that diiodotyrosine partially inhibited the suppressing effect of the acid salt of auric chloride on oxygen uptake by saliva-sugar mixtures. This fact stimulated a series of experiments to test whether the combination of diiodotyrosine with a gold chloride solution might form an interaction detectable by spectrophotometric technics. It was soon found that such a combination in the presence of starch led to the formation of a pink color in the mixed solutions. This color development proceeded very slowly at 37° C. but could be hastened by placing the mixture in a boiling water bath. The following metallic ions were combined with diiodotyrosine and starch in the same proportions. Au^{+++}, Pt^{++++}, Pd^{++}, Pb^{++}, Hg^{++}, Cu^{++}, Cu^{+}, Ag^{+}, Al^{+++}, Cd^{++}, Mg^{++}, and Mn^{++}. Only the Au^{+++} gave the characteristic pink-colored reaction. Work is now being undertaken to develop this test into a qualitative and quantitative means of detecting gold in the presence of the other ions usually interfering in detecting and estimating procedures. Also, there is a possibility that this reaction may be utilized as a test for the presence of diiodotyrosine and related compounds in body fluids.

T262. Removal of Radioactive Fat and Protein From the Mouth.—*W. W. Wainwright, Los Angeles, F. K. Bauer and P. B. Thomas, University of Southern California School of Medicine, Los Angeles.* Peanut butter was mixed with 2 μc of radioiodinated (1) human serum albumin or (2) triolein. Five healthy subjects had their thyroid glands blocked with 10 drops of Lugol's solution on the day of the experiment and for several days later to prevent uptake of radioactive iodine from the metabolized test substances. A wide-angle scintillation counter tube was pointed toward the oral cavity through the cheek of the test subject. The test subject then put a soda cracker covered with the radioactive peanut butter in his mouth and the radioactivity was measured. The background of the instrument was subtracted from the counts of radioactivity obtained and the net counts were expressed as 100 per cent since the entire test meal was measured. Forty-eight technically satisfactory experiments were carried out. Results: (1) 4 to 33 per cent (mean 18 per cent) of the radioactive test meal remained in the oral cavity following completion of chewing and swallowing the cracker. (2) After rinsing the mouth, two thirds to one half of the remaining radioactivity disappeared. In 2 experiments the mouth was clear after rinsing. (3) In the others, background level of radioactivity was reached in 5 to 10 or more minutes. (4) After rinsing and chewing one stick of gum for 1 minute, mouth was clear after 1 minute in 17 experiments except 1 (five minutes) and for the 2 immediate clearances mentioned in (2). (5) After rinsing and 1 minute of brushing the mouth was clear within 3 minutes in all test subjects.

T263. Effect of Fluoride on the Developing Teeth of Rats.—*B. R. Bhussry, University of Rochester, Rochester, N. Y., and Georgetown University, School of Dentistry, Washington, D. C.* It is the purpose of this report to show the effects of certain concentrations of F^- on the development of teeth in rats. In the present experiments, a total of 50 young rats, 3 to 5 days old, was used.

A dose of 0.1 ml. and 0.2 ml. of fluoride stock solution (1,000 ppm) was administered to them daily either by drinking water or by intraperitoneal injection. Adequate series of controls were maintained. All animals were given a non-caries producing diet and distilled water. They were weighed biweekly for the duration of the experiment. The animals were sacrificed after 5, 10, 15, 20 doses, respectively. The jaws containing developing teeth were fixed in Bouin's solution, decalcified by Morse's technic, embedded in paraffin, sectioned and stained with hematoxylin and eosin and Masson's trichrome stains. The observations so far reveal that the changes in the incisor teeth are more marked than those in the developing molars. The walls of the ameloblasts show considerable thickening and tend to become disorganized with increased doses of fluoride. Their cytoplasm is pale staining and shows the presence of vacuoles. The nucleoli disappear and the nuclear chromatin becomes clumped. The odontoblasts do not show any significant changes. The increased vascularity of the pulp is indicated by the blood vessels becoming engorged with red blood cells.

T264. Ascending Pulpitis in Primary Teeth.—*Viktors Kalnins, School of Dentistry, Western Reserve University, Cleveland, Ohio.* Radiographic and histologic examination was performed on 102 human primary teeth. In the course of the establishment of a mixed dentition, primary canines and second primary molars can be deprived of their alveolar bone alternately on the mesial and distal aspects for a long period before exfoliation of these teeth takes place. This occurs when the erupting neighboring permanent tooth exerts intensive pressure on the adjacent wall of the primary alveolus, causing its partial or complete destruction. Later, when eruption of the neighboring permanent tooth is accomplished, the primary tooth is supported by a new alveolar wall which is part of the permanent alveolus. Destruction of the alveolar bone associated with the remolding of the alveolus, oblique or unilateral resorption of roots in canines, and resorption of only one root in molars may provoke an apical exposure of the primary pulp by way of a gingival pocket with subsequent development of an ascending pulpitis. The pulpitis is usually granulomatous in type and is accompanied by osteoclastic resorption of the inner walls of the root, which radiographically appears as internal resorption. When the apical pulp exposure becomes occluded, due to development of the adjacent permanent alveolus, spontaneous healing of the ascending pulpitis may take place. In the treatment of the primary teeth by pulpotomy or capping, healing of the pulpal wound may be disturbed due to coronal spread of the ascending pulpitis.

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